

For Reference

NOT TO BE TAKEN FROM THIS ROOM

PHOSPHORUS METABOLISM OF THE
ADRENALS, LIVER, AND TUMOR OF RATS
BEARING THE WALKER 256 CARCINOMA

D. C. Hobbs

Ex LIBRIS
UNIVERSITATIS
ALBERTAENSIS





Digitized by the Internet Archive
in 2018 with funding from
University of Alberta Libraries

<https://archive.org/details/Hobbs1955>

ABSTRACT

A number of the systemic changes produced in tumor-bearing animals have been reviewed in relation to an involvement of the pituitary-adrenal system. They might be separated into a) those which can be produced by adrenal cortical hyperfunction, and b) those which might result from a hypofunction.

A study was made of the P^{32} incorporation into various P-containing fractions of the adrenal glands of animals bearing the Walker 256 carcinoma, since previous work involving hypophysectomy and cold stress has indicated some degree of correlation between P^{32} uptake in the adrenals and the activity of these glands.

The P fractions studied were: plasma inorganic P, the inorganic P, ATP P, total acid soluble P, lipid P, RNA P, and DNA P of the liver and tumor. The same fractions, with the exception of DNA P, were studied in the adrenal tissue. A study was made of the changes in P^{32} incorporation at various time intervals after P^{32} administration and after tumor innoculation.

A decreased incorporation of P^{32} into the adrenal fractions was noted and it was postulated that this was

due to a defect in the passage of P^{32} from the extracellular to the intracellular fluid space, similar to the condition found in hypophysectomy.

It was further postulated that the decreased P^{32} incorporation in tumor-bearing animals is associated with a lowered output of cortical hormones which, in turn, is responsible for some of the systemic changes noted in the host.

The administration of cortisone and ACTH to tumor-bearing animals was ineffective in restoring the P^{32} incorporation into the P-containing fractions of the adrenals or liver.

A greatly increased rate of incorporation of P^{32} into the liver DNA of tumor-bearing animals was also noted.

Thesis
1955
#12.

THE UNIVERSITY OF ALBERTA

PHOSPHORUS METABOLISM
OF THE ADRENALS, LIVER, AND TUMOR
OF RATS BEARING THE WALKER 256 CARCINOMA

A DISSERTATION
SUBMITTED TO THE SCHOOL OF GRADUATE STUDIES
IN PARTIAL FULFILMENT OF THE REQUIREMENTS FOR THE DEGREE
OF MASTER OF SCIENCE

SCHOOL OF PHARMACY

by
DONALD CLIFFORD HOBBS

EDMONTON, ALBERTA,

APRIL, 1955

This investigation was made possible through a grant from the National Cancer Institute to Dr. B. E. Riedel. The work was carried out in the McEachern Cancer Research Laboratory.

ACKNOWLEDGMENTS

The writer wishes to acknowledge the assistance and cooperation rendered by others. The problem was suggested by Dr. B. E. Riedel whose guidance and suggestions throughout the course of the investigation were of great assistance.

The Walker 256 carcinoma was supplied by Dr. A. G. Stewart whose suggestions were also appreciated. The histological sections were prepared by Mr. B. Fairall and examined by Dr. T. Shnitka.

CONTENTS

	Page
Abstract	ii
Acknowledgments	v
List of Illustrations	x
INTRODUCTION	1
A. The Tumor-Host Relationship	1
B. The Application of Radioactive Isotopes	4
HISTORICAL BACKGROUND	7
A. The Walker Carcinoma	7
B. Nature of the Systemic Effects	11
C. The Relationship between the Tumor and the Endocrine System	13
1. Adrenal Changes in Tumor-Bearing Animals	14
2. Systemic Changes in the Host	16
3. Action of the Endocrine system on the Tumor	22
D. The Effects of Stress on the Adrenals ..	25
E. Metabolism Studies of the Liver	28
F. Metabolism Studies of the Tumor	30
METHODS	33
A. Animals	33

	Page
B. Injections	35
C. Tissues	36
D. Separation of the P Compounds	36
1. Plasma Inorganic P	37
2. Acid Soluble P	37
3. Inorganic P	37
4. Adenosine Triphosphate (ATP) P	39
5. Total Acid Soluble P	39
6. Lipid P	39
7. Nucleic Acid P	40
E. Phosphorus Estimation	40
F. Radioactivity Determination	42
G. Definition of Terms	42
H. Statistical Analysis	43
I. Autoradiography	43
RESULTS	45
A. The Effect of Time after P ³²	45
1. Plasma	47
2. Adrenals	49
(a) Inorganic P	49
(b) Total Acid Soluble P	49
(c) Lipid P	51
(d) RNA P	51
(e) DNA P	51
3. Liver	53
(a) Inorganic P	53
(b) Total Acid Soluble P	55

	Page
(c) Lipid P	55
(d) RNA P	57
(e) DNA P	59
3. Tumor	60
(a) Inorganic P	60
(b) Total Acid Soluble P	60
(c) Lipid P	60
(d) RNA P	62
(e) DNA P	62
B. The Effect of Time after Tumor Inoculation.	65
1. Plasma	65
2. Adrenals	65
3. Liver	68
4. Tumor	68
C. Effects of Cortisone and ACTH	71
1. Plasma	71
2. Adrenals	71
3. Liver	71
D. Autoradiographs	73
DISCUSSION	74
A. Plasma	75
B. Adrenals	76
C. Liver	80
D. Tumor	81
SUMMARY	83
Bibliography	85
Appendices	following 92

LIST OF ILLUSTRATIONS

Figure	Page
1. Walker 256 carcinoma (x50)	9
2. The Walker 256 carcinoma after 20 days of intramuscular tumor growth	34
3. Excised Walker 256 carcinoma after 20 days of intramuscular tumor growth	34
4. General separation scheme for the P-containing compounds	38
5. The relationship between optical density at 730 m μ and concentration of P	41
6. The change in the corrected specific activity of the inorganic P of the plasma with time after the administration of P ³² for normal (N) and tumor-bearing (T) animals	48
7. The change in the relative specific activity of the inorganic P of the adrenal with time after administration of P ³² for normal (N) and tumor-bearing (T) animals	50
8. The change in the relative specific activity of the total acid soluble P of the adrenal with time after administration of P ³² for normal (N) and tumor-bearing (T) animals	50

Figure	Page
9. The change in the relative specific activity of the lipid P of the adrenal with time after administration of P^{32} for normal (N) and tumor-bearing (T) animals	52
10. The change in the relative specific activity of the RNA P of the adrenal with time after administration of P^{32} for normal (N) and tumor-bearing (T) animals	52
11. The change in the relative specific activity of the inorganic P of the liver with time after administration of P^{32} for normal (N) and tumor-bearing (T) animals	54
12. The change in the relative specific activity of the total acid soluble P of the liver with time after administration of P^{32} for normal (N) and tumor-bearing (T) animals	56
13. The change in the relative specific activity of the lipid P of the liver with time after administration of P^{32} for normal (N) and tumor-bearing (T) animals	56
14. The change in the relative specific activity of the RNA P of the liver with time after administration of P^{32} for normal (N) and tumor-bearing (T) animals	58
15. The change in the relative specific activity of the DNA P of the liver with time after administration of P^{32} for normal (N) and tumor-bearing (T) animals	58

Figure	Page
16. The change in the relative specific activity of the inorganic P of the tumor with time after administration of P^{32}	61
17. The change in the relative specific activity of the total acid soluble P of the tumor with time after administration of P^{32}	61
18. The change in the relative specific activity of the lipid P of the tumor with time after administration of P^{32}	63
19. The change in the relative specific activity of the RNA P of the tumor with time after administration of P^{32}	63
20. The change in the relative specific activity of the DNA P of the tumor with time after administration of P^{32}	64
21. The change in the corrected specific activity of the inorganic P of the plasma with time after tumor inoculation	66
22. The change in the relative specific activities of the inorganic P and total acid soluble P of the adrenals with time after tumor inoculation	67
23. The change in the relative specific activities of the lipid P and RNA P of the adrenals with time after tumor inoculation	67

Figure	Page
24. The change in the relative specific activities of the inorganic P, total acid soluble P, and lipid P of the liver with time after tumor innoculation	69
25. The change in the relative specific activities of the RNA P and DNA P of the liver with time after tumor innoculation	69
26. The change in the relative specific activities of the inorganic P, total acid soluble P, lipid P, RNA P, and DNA P of the tumor with time after tumor innoculation	70
27. The effect of Cortisone and ACTH on the relative specific activities of the adrenal tissue fractions	72
28. The effect of Cortisone and ACTH on the relative specific activities of the liver tissue fractions	72

INTRODUCTION

A. THE TUMOR-HOST RELATIONSHIP

A tumor and the organism within which it grows may be considered as two separate entities - a dual organism. The tumor represents a parasite within the animal; it derives sustenance from, but is not under the control of, the host. Once they have attained their full malignancy the tumor cells appear to be beyond the normal regulative controls exercised by the organism and continue to grow, heedless of the well-being of the host.

However, the tumor itself is unable to obtain food from the outside or to dispose of waste products; it therefore elaborates an extensive blood supply network from the unwilling host. Once this is accomplished, the tumor is free to grow so long as the host can continue to supply metabolites and oxygen, and to dispose of its waste products.

In some instances the tumor mass will interfere mechanically with some vital function of the organism and cause death. Nevertheless, in the absence of any such anatomical effect, the host will usually die long before its nutritive capacities are exceeded. Although the host often exhibits a marked loss in weight (10), pair-feeding has shown that

the animal does not die of simple inanition (69). Nor will force-feeding substantially prolong its life (12, 100).

The tumor must exert some specific influence on the host which is transmitted through the common blood supply of the dual organism. The production of such effects is known as the tumor-host relationship.

Begg (10) has defined the systemic effects of a tumor as "the changes produced in the host which are remote from the tumor and in which no evidence of metastatic malignant cells is found." These effects are extensive, involving many distant and unrelated organs. It is remarkable, then, that there is an astonishing similarity in the systemic effects produced by widely differing tumors. The tumor appears to be more closely related to other tumors than it is to the tissue of origin within the host, or to any other normal tissue. As it is successively transplanted the tumor becomes more and more like other tumors and less and less like any other organ of the body. In addition, the effects produced by various types of tumors will be similar from one species to another. The effects are not due to the mere presence of rapidly growing tissue since embryonic tissue will not have the same result, though it may grow at the same rate (31).

Most, if not all, of the systemic effects may also occur in non-cancer conditions. This is unfortunate since it lessens the possibility of finding a specific test for cancer.

For example, the depression of liver catalase activity noted in tumor-bearing hosts may also be produced by acute starvation (6, 21, 70) and adrenalectomy (13), and liver enlargement has also been noted in pregnancy (80). Similar situations exist for many of the other changes noted in the tumorous animal. However, no single non-cancer condition will produce all of these effects.

Thus it is unlikely that any new reactions have been initiated or normal reactions abolished in the tumor-bearing animals. All changes probably result from quantitative alterations in reactions already in progress. Stetten (98) has surmised that all reactions are spontaneous and take place without any outside stimulation. They do not necessarily proceed at their maximal rate in the normal animal, being responsive to both inhibitory and stimulatory control. (He compares this to the duality of control of the autonomic nervous system.)

The controlled reaction in the normal animal may find itself out of control in the tumor-bearing animal due to either an increase or decrease in the inhibitory or stimulatory component. A study of the rates of these reactions, then, would assist in elucidating the underlying causes of the changes shown in the particular system, since all changes in any organ must be results of changes in one or more chemical reactions. The altered reaction rate, in turn, is indicated by altered rates of formation or removal of the

products and precursors.

B. THE APPLICATION OF RADIOACTIVE ISOTOPES

The use of radioactive isotopes makes it possible to "label" certain atoms and follow their course within the body. The organism is unable to distinguish the molecule containing the labelled atom from the normally-occurring form and thus proceeds to treat it in the same manner as any other molecule of that compound. After a definite time interval the tissue may be fractionated and the amounts of radioactivity determined. The concentration of the radioisotope within the particular compound is an indication of the rate at which the tissue is incorporating that atom into the compound. Since the body is in a dynamic state, the activity of the substance will also depend upon the rate at which it is being destroyed.

By this means it is unnecessary to produce drastic changes in the steady state in order to make a chemical determination of a particular compound. By ordinary analytical methods it is only possible to determine the concentration of a particular substance within the tissue. By the use of radioactive tracers it is also possible to determine the amount of that compound which has been manufactured anew over the particular time interval, and the amount that was already present before the introduction of the tracer into the body.

Before a radioactive isotope can be used in the study

of metabolic changes, certain criteria must be satisfied (54):

1. The initial concentration of the tracer must be sufficient to withstand dilution during metabolism.
2. Throughout metabolism the label must adhere to the particular molecule or portion of molecule with which it is originally associated. There must be no direct exchange reactions, but only those reactions which are produced as a part of the normal metabolic mechanism of the organism.
3. Abnormalities in metabolism must not be brought about through the action of the isotopic sample on the organism.
4. The half-life of the isotope used must be sufficiently long so that decay does not remove the tracer faster than it can be extracted, characterized, and assayed.

Radioactive phosphorus (P^{32}) is particularly useful in biochemical studies since phosphorus (P) is involved in a major mechanism for the storage and utilization of energy for synthesis. A study of the rates of P^{32} incorporation into the various tissue fractions is of great significance since they are indicative of the speed at which the various compounds are phosphorylated in their preparation for further catabolism and anabolism. These various P-containing compounds may be fractionated after removal from the tissue and a study of the amount of P^{32} incorporated into them is indicative of the rate at which they are being formed and destroyed.

After injection, the P^{32} quickly finds its way into the blood stream which is in rapid isotope equilibrium with the extracellular fluid (38). From here it passes somewhat more slowly into the intracellular fluid (85). The various enzyme systems of the cell which are acting on the compounds in the intracellular fluid to form other compounds cannot distinguish the radioactive from the non-radioactive molecule. The product formed at that moment will have a radioactive isotope concentration identical to that of the precursor. The concentration in the precursor is constantly changing and thus the concentration in the product being formed will also be constantly changing.

Zilversmit et al (117) have shown that the true turnover rate of a compound cannot be determined without knowing the immediate precursor and its activity. In most cases the precursor is unknown. However, much valuable information can be gained by comparing tumor against normal animals. Although the true rate of incorporation is unknown, changes can be detected.

The purpose of the research reported in this thesis was to compare the P metabolism, as indicated by the uptake of radioactive P, of rats bearing the Walker 256 carcinoma with normal rats. In addition the effect of ACTH and Cortisone on the P metabolism was briefly examined. The tissues used for fractionation and examination were the adrenals, liver and, in tumor animals, a sample of the tumor.

HISTORICAL BACKGROUND

A. THE WALKER CARCINOMA

The Walker 256 carcinoma arose as a mammary adenocarcinoma which was noticed on the abdomen of a pregnant female rat by Dr. G. Walker in 1928 (93). After removal of the mass, portions were implanted into 16 young rats. Of these 16 original rats, 2 died shortly without growth of tumor and 5 continued to live without tumor growth. The remaining 9 showed tumors. Subsequent implantations of these tumors showed a rise in the percentage of tumors and, after a few generations, the percentage of "takes" was nearly one hundred. There was an extremely low incidence of regression. Dr. Walker continued to carry the tumor in his laboratory for four years, transplanting it approximately every two weeks. Numerous rat strains were investigated and all showed a high percentage of takes.

At present, after numerous generations of transplantation, the tumor has changed from its original form as described by Dr. Walker and has become stabilized in its characteristics. It consists of smooth rounded tissue masses encapsulated by a well-defined fibrous capsule. Beneath the thin capsule is a zone of healthy actively-growing tumor tissue of a translucent white color. Where

the tumor is only a few days old, this tumor tissue comprises all of the material within the capsule; in older tumors there is a core of dark necrotic tissue of a semi-fluid consistency. Where the tumor is very old (3 to 4 weeks) the necrotic tissue forms almost the entire mass of the tumor. A few instances have been noted of young small tumors which were largely necrotic and of large old tumors which had a disproportionate amount of healthy tumor tissue.

Histological examination (Fig. 1) shows tumor tissue infiltrating between fasciculi and fibres of voluntary muscle. It is composed of sheets of anaplastic epithelial cells, supported by a scant, relatively avascular fibrous stroma. The latter is disposed around clumps of cells, as well as between individual cells. A dominant cell type, showing considerable variability in size, shape, and staining properties, can be recognized. This is of large size, and polygonal, circular, or fusiform in outline. It contains a large, vesicular, centrally-placed nucleus, with prominent nucleolus and nuclear membrane. Only moderate cytoplasmic basophilia is evident. Three to five mitotic figures are noted per high power field, and occasionally these are of bizarre form. Tumor giant cells are of infrequent occurrence, as are inflammatory cells.

The tumor may be transplanted either subcutaneously or intramuscularly. The latter method was used in this inves-



Fig. 1. Walker 256 carcinoma (x50).

tigation as it seemed to give a more uniform rate of growth from animal to animal (103). The tumor tissue may be implanted by means of a trochar or may be coarsely homogenized and injected in saline.

Many advantageous properties commend the use of the Walker 256 carcinoma in cancer investigation.

1. It is easily transplanted and the percentage of takes is almost 100. Regression is rare.
2. The growth is rapid and uniform and allows study over a short time period.
3. After numerous generations its characteristics have become stabilized and a definite course may be expected to ensue in the animal.
4. It is similar in many characteristics to other experimental and natural tumors, allowing a comparison with the results found in investigations using other tumors.
5. The tumor represents all of the important biological and biochemical properties of malignant tissue.
6. Systemic changes must be due to the tumor and cannot be due to any substance administered to induce a tumor.

One disadvantage of the Walker (or any transplantable) tumor is that there are certain differences from spontaneously occurring tumors (extreme malignancy and anaplasia, few metastases, rapid growth, extreme necrosis, etc.). However, the physiological, anatomical, and biochemical effects on the host are similar.

B. NATURE OF THE SYSTEMIC EFFECTS

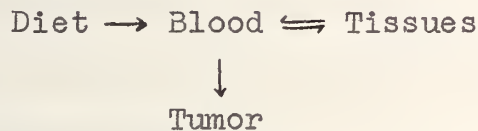
Much experimental work has been carried out in an effort to determine the method by which the tumor produces the systemic effects. Since the tumor is associated with the host only by a common blood supply, it has been postulated that a) the tumor produces some toxin which, circulating in the blood of the host, gives rise to the altered metabolism of its organs, or b) the tumor extracts some substance from the blood giving rise to a deficiency in the host.

The toxin theory is based largely on one of the most pronounced and reproducible effects of malignant tumors, that of reduction of the catalase activity of the liver. Greenstein and his associates have studied this problem extensively. The degree of depression is related to the size of the tumor (1) and returns to normal following excision of the tumor (33). Embryonic tissue will not produce the reduction (31), indicating that the presence of rapidly growing tissue is not responsible. However, acute starvation will elicit the change (70).

On the premise that a toxin is responsible, considerable work has been carried out on the extraction from tumors, and injection into normal animals, of crude and prepared materials. An active substance has been obtained from human tumors by Nakahara and Fukuoka (74, 75) and from mouse tumors by Greenfield and Meister (27, 28).

These semi-purified extracts, which will produce the typical lowering of catalase activity, have been the basis of numerous chemical analyses but the exact composition of the active agent is not yet known. Evidence has pointed to a proteose or polypeptide (75).

The deficiency theory is supported by studies of nitrogen balance. The tumor appears to extract nitrogen from its blood supply in amounts which are much greater than that demanded by normal tissue. The body attempts to keep the blood concentration at a fixed level and, when the dietary supply is insufficient, protein is extracted from the tissues of the host.



The interchange between the blood and tumor is not reversible as is the case between the blood and tissues. Thus the tumor has been likened to a "nitrogen trap." The elimination of nitrogen from the diet will only slow the rate of growth of the tumor however (51). Maintenance of the dietary protein intake by force-feeding (99) will not eliminate the systemic effects although the loss of carcass weight (animal minus tumor) is practically eliminated.

Therefore the tumor acts in a greater capacity than as a simple nitrogen trap to produce these effects. It is possible that some other essential metabolites are removed

as well as protein, and to such a degree that force-feeding will not overcome the deficiency. Or perhaps the removal of some substances is combined with the production of toxins. It is hoped that further work will answer these questions.

Whether the toxin and/or deficiency theories apply, the question also arises as to whether the systemic effects are primary or secondary. The tumor may have an action on one (or more) organs which, in turn, mediate the effect to the other tissues. It has been suggested (20) that an influence on the adrenal gland might be a primary effect, hypofunction of the cortex producing some of the secondary effects. That adrenal hypofunction could produce at least some of these effects has been shown. Conversely, some of the systemic effects are similar to the changes produced by adrenal hyperfunction.

C. THE RELATIONSHIP BETWEEN THE TUMOR AND THE ENDOCRINE SYSTEM

A study of the P uptake of the various fractions in the adrenal gland and the effects of a distant tumor on this organ involves an examination of the interrelationships between the tumor, the adrenal gland, and the rest of the body. The tumor has its effects on the whole body; the mechanism of action is embodied in the two theories previously outlined. The body attempts, unsuccessfully, to throw off this foreign organism; the pituitary-adrenal

system probably manifests the general adaptation syndrome of Selye (95).

The theory that the adrenals are primary target organs and mediate some of the systemic effects necessitates a consideration of the changes produced in the adrenals of the tumor-bearing animal, the systemic changes produced in the body, and how the two may be related. Of interest also would be the influence of the pituitary-adrenal system itself on tumor growth.

1. Adrenal Changes in Tumor-Bearing Animals

The changes found in the adrenals are analogous to those of exhaustive hypofunction, as described in the Type III adrenal response of Sayers (88).

It was first noticed that some of the symptoms of the terminal stages of cancer were similar to those of Addison's disease in humans (87) and adrenal insufficiency in experimental animals. McEuen and Selye (64) found that rats bearing the Walker 256 carcinoma contained areas of infiltrated lymphocytes and other leucocytes in the adrenals but only where the tumor weighed more than 50 grams. They concluded that it was due to secondary infection. Ball and Samuels (8) noticed hypertrophy of the adrenals due to an increase in cytoplasm and in the number of cortical cells. They also noted an increased lipid content and found that the adrenal changes could be prevented by hypophysectomy.

Saranson (87) found that cancer patients dying in a cachectic state had greatly enlarged adrenals showing varying degrees of lipid depletion, whereas moderately enlarged adrenals and a normal lipid pattern were found in those cancer patients dying of cancer but with little weight loss.

Using transplantable tumors in mice, Dalton and Peters (20) could find no increase in adrenal weights. In a histological study, however, they found a decrease in stainable lipid beginning at the inner border of the zona fasciculata and extending, in the most striking cases, to the zona glomerulosa. They compared it to the effects of moderate to acute pituitary stimulation.

Haven et al (36) using the Walker 256 carcinoma, made a chemical study of the adrenal lipids. They found a decrease in the total steroid and cholesterol levels in all tumorous animals and a lowered concentration of fatty acids and total lipids where the tumors were over 30% of the body weight. They concluded that, as the steroids were depleted, their place was taken at first by fat and, as the animals became more emaciated, the fat was used up. The observation was also made that, although the steroid concentration of the adrenals was decreased, the amount per 100 grams of rat plus tumor remained approximately the same. Cholesterol levels are of interest, since it is believed that cholesterol is a precursor of the adrenal steroids (61).

The adrenal ascorbic acid level has also been studied. A marked decrease has been noted (89) which appears to be related directly to the size of the tumor.

Stewart and Begg (100) found that the force-feeding of rats which were carrying the Walker 256 carcinoma reduced but did not completely eliminate the carcass weight loss. If the adrenal hypertrophy had been produced by inanition, restoration of nitrogen would be expected to decrease the adrenal hypertrophy. On the contrary, there was a greater hypertrophy in the force-fed tumor-bearing rats than in the tumor rats fed ad libitum. They pointed out, however, that this was probably due to the stress of the force-feeding itself. It has been shown that feeding a high-protein diet will also produce adrenal enlargement (46, 106).

2. Systemic Changes in the Host

On the basis of the changes in the adrenals of the host it has been suggested that there is an exhaustive hypofunction of the glands. This is supported by many of the changes noted in the tumor-bearing animal, some of which can be produced by adrenalectomy and some of which can be alleviated by cortisone administration.

One of the most striking systemic effects is the decrease of liver catalase activity (1, 29, 32). The nature of this decrease and the evidence indicating that it is a direct effect of the tumor has been commented upon pre-

viously. This effect probably could not be mediated entirely through the adrenals, since the degree of reduction produced by adrenalectomy (13) is not as great as that found in the tumor-bearing animal. The tumor extract obtained by Greenfield and Meister (28) produced a marked catalase activity reduction in normal mice after a single injection. However there were no changes noted in other parts of the body, including the adrenals.

Anaemia is another factor which is noted in tumor-bearing animals (105). White and Dougherty (113) have shown that continuous injection of ACTH results in an increased hemoglobin level and that adrenalectomy will reduce the level. This is also supported by the numerous stress agents which will act on the pituitary-adrenal system to raise the hemoglobin level. In the tumor-bearing animal there is probably a similar stress effect but the hemoglobin level is decreased rather than increased.

The changes produced in the thymus, lymph nodes, and other lymphoid tissues by tumors are particularly interesting since these organs are intimately related to nitrogen metabolism. One of the effects of inanition is to produce an involution of the lymphoid tissue with loss of nitrogen (2). This mobilization of nitrogen is dependent upon the pituitary-adrenal system, since it is absent in hypophysectomized and adrenalectomized animals (114). Cortical extracts will cause thymus atrophy in both hypophysectomized and intact rats (45) and ACTH has the same effect (71).

An involution of the lymphoid organs has also been noted in the tumor-bearing animal (10, 73). This lymphoid involution might be a direct effect of the action of the tumor in growing at the expense of the host. There is extensive protein depletion in the host but no increase in the excretion of nitrogen; all of the protein loss results from a movement of nitrogenous substances from normal to neoplastic tissue (96). That this is a one-way passage is indicated by the slow decline of the concentration of labelled amino acids in the tumor (34). It is suggested that the host responds to this decreased blood nitrogen level by removing nitrogen from the tissues (66) when there is insufficient in the diet, the action being controlled by the pituitary-adrenal system.

Maintenance of the food intake by force-feeding (12, 100) maintains the carcass weight and there is little nitrogen lost in the host. In these animals, however, there is no diminution of the other systemic effects such as adrenal hypertrophy, anaemia, loss of liver catalase activity. Begg and Dickenson suggest (12) that the loss of carcass weight in tumor-bearing animals is not a necessary component of the reaction leading to the development of systemic effects. This would indicate that the changes in the adrenal are not due to inanition.

Savard (90) also found thymus involution in tumor-bearing mice but noted a hyperplasia of the lymph nodes.

This latter effect was also noted by Homburger (42). Both of these workers used the Sarcoma 180 in mice. Murphy and Sturm (72) noted an involution of the cervical lymph nodes in rats bearing a lymphosarcoma. This apparent discrepancy may be due to a species difference as suggested by Stewart (99). Experiments with adrenalectomized animals show that the thymus atrophy in tumor-bearing animals is dependent upon the adrenals in the case of the rat (99) but not in the case of the mouse (90).

Some of the evidence would seem to indicate that the thymic involution in tumor-bearing animals is mediated through the pituitary-adrenal system and conforms to the general adaptation syndrome (95). Selye (94) found a typical involution of the thymus mediated through the pituitary-adrenal system following the administration of a number of drugs. Upon prolonged administration the thymus returned to normal, indicating an "alarm reaction." This is in contrast to the ever-increasing thymus involution noted in tumor-bearing animals where the effect on the thymus is dependent on the size of the tumor (10). It could be concluded that the effect of the tumor in part directly on the lymphoid glands and is not necessarily entirely due to an adrenal hyperactivity.

It has been shown that hyperthyroidism will also produce alterations in the size of the lymphoid structures (114) and an action of the tumor mediated through this organ might be postulated. This question would bear investigation.

Another property of malignant tumors that has been related to the pituitary-adrenal system is the reduced ability to synthesize glycogen in the liver. Young et al (115) showed that patients with gastric cancer were unable to transform glucose given by stomach tube into hepatic glycogen at a normal rate. This defect was corrected by injection of adrenal cortical extract but was not similar to the situation in adrenalectomized animals or in patients with Addison's disease where the blood sugar was lowered by fasting.

Using mice bearing Sarcoma 180, Young et al (116) found an effect similar to that found in patients bearing gastric cancer. Following a 16 hour fast the liver glycogen contents in both normal and tumor mice was found to be the same; however in the fasted animals given an intraperitoneal injection of glucose the glycogen level in the tumor animals was only about 60% of that in the control animals. This decreased ability to synthesize glycogen could not be explained on a nutritional basis, since the food intakes and body weights were similar.

This abnormality in the livers of tumor-bearing animals suggests a decreased activity of the adrenal glands. It has been shown (62, 107) that adrenalectomy will produce an inability to synthesize glycogen, which can be restored by the administration of Cortisone.

Hyperlipemia, which has been noticed in the tumorous animal (36, 37, 99), may be related to hyperfunction of the

adrenal glands. The administration of large doses of Cortisone has resulted in lipemia in rats (49, 99) but the same effect could not be produced by ACTH (50, 99). Haven et al have suggested, on the other hand, that the hyperlipemia in tumor rats may be due to an increased caloric requirement in the tissues (36) or a demand in the tumor for unsaturated fatty acids needed for the formation of phospholipid and cholesterol esters (37). That the lipids remain within the dual organism is indicated by the fact that there is no ketonuria or increase in the titratable acidity of the urine in tumor-bearing rats (68). The fat which is not stored must be burned.

The evidence accumulated to date is very controversial regarding the effect of the tumor on the adrenals, the adrenals on the host, and the systemic changes on the adrenals. In some cases a typical stress reaction appears to be indicated, other changes suggest a specific effect of some change in the body on the pituitary-adrenal system. A direct effect of the tumor on the adrenals has been suggested but not proven.

Hypofunction of the adrenals may be partially responsible for the anaemia, diminished catalase activity and lowered glycogen-synthesizing ability in the liver found in tumor-bearing animals. It cannot produce the effects alone, since adrenalectomy has been shown to produce only

half as great a catalase activity reduction (13) and only one third the decrease in hemoglobin level (113). The changes within the adrenal gland itself (increased weight, decreased ascorbic acid and cholesterol) are remarkably similar to exhaustive hypofunction as exemplified in the Type III adrenal response of Sayers (88) during "intense continuous stress ending in death."

The hyperlipemia and thymus atrophy might seem to point to hyperfunction of the adrenal glands.

There is undoubtedly a complex of interreactions taking place between the three components: tumor, host, and pituitary-adrenal system. Nevertheless, the adrenals cannot be both hyperfunctioning and hypofunctioning at the same time. One solution of this controversy would lie in the direct measurement of the level of circulating cortical hormones. This, however, is impossible until a more sensitive analytical procedure is available. Another means of examining cortical function with an eye to postulating a possible effect on corticoid output, although it has not been conclusively proven to be directly related to hormone production, would be to measure the rate of turnover of the various P-containing fractions in the gland. Such a procedure is one of the purposes of this investigation.

3. Action of the Endocrine System on the Tumor

Adrenalectomy has been shown to retard the growth of human (43) and transplanted rat (23, 47, 104) tumors, while Sturm and Murphy (101) report that there is an increased

growth of certain lymphoid tumors. Talalay et al (104) have shown that the reduced tumor growth following adrenalectomy can be counteracted only slightly by the administration of cortisone.

Hypophysectomy has also been studied in its effects on tumor growth. McEuen and Thompson (63), using the Walker tumor, found a decreased rate of tumor growth in hypophysectomized animals. However, they also found they could produce just as great a retardation of growth by dietary restriction in the animal and thus concluded that there was merely a non-specific effect of hypophysectomy on weight. Loefer (60) also noted a decreased tumor growth relative to body weight in hypophysectomized animals, which became an increased growth rate when related to body weight increase. The percentage of "takes" was not affected. Samuels and Ball (86), using the Walker tumor, found a decreased incidence, while Loeb and Kirtz (59) found an increased incidence when pituitary tissue implantation was used in conjunction with the tumor.

Ball and Samuels (7), using the Walker rat tumor, tube-fed a controlled amount of diet to hypophysectomized animals and restricted the control animals so that the body plus tumor weight remained constant. They found a greatly retarded tumor weight (about one third) in the hypophysectomized animals as compared to normal animals. The hypophysectomized tumor rats, however, were force-fed more than their normal intake and the control animals received less

food than they would normally have consumed, making conclusions regarding the effect of hypophysectomy obscure.

The function of the hypophysis in maintaining the adrenal is well-known (18), but the effects of hypophysectomy on tumor growth may not be mediated entirely through the adrenals since Talalay et al (104) have shown that the effects of simultaneous adrenalectomy and hypophysectomy are cumulative.

The action of hypophysectomy in retarding tumor growth and of pituitary extract administration in accelerating it may be due to the action of growth hormone, not ACTH, as it has been shown (97) that the administration of pure growth hormone will increase the tumor growth and body weight in mice bearing a mammary adenocarcinoma. The metabolic rate, however, is not a factor since Samuels et al (86) have shown that there is the same retardation of tumor growth in hypophysectomized rats regardless of whether thyroid substance was administered.

The effects of administration of hormones to intact tumor animals has also been the subject of much investigation. Varying reports have been made of the effect of cortisone on the growth of tumors. Tumors of lymphatic origin show slight temporary inhibition (17, 48, 102) as do some other types of transplantable tumors (15, 102). Others report no effect of cortisone on various mammary adenocarcinomas (26, 102). Begg (11) and Talalay et al (104) found no effect on the Walker tumor, although Ingle et al (51) report an inhibition of

growth. Baserga and Shubik (9) found a similar inhibition in a transplanted mouse mammary adenocarcinoma at the 10 day period but the tumor then proceeded to grow at a rate faster than normal and soon reached the size found in the control tumor animals. Thus, they point out, the inhibition noted by Ingle et al (51) may be due to too early a sacrifice of the animals. In general, it would appear that cortisone inhibits lymphoid tumors but not those of an epithelial origin.

Although the administration of ACTH increases the cortical secretions, numerous workers have reported negative effects of ACTH on both lymphoid and epithelial tumors (17, 26). An exception is the regression of human lymphoid tumors reported by Pearson et al (79).

D. THE EFFECTS OF STRESS ON THE ADRENALS

In view of the controversial nature of the changes produced in the adrenal glands of the tumor-bearing host, it is surprising that so little work has been done on the P metabolism in this organ. Some studies have been made of the effect of stress on the adrenals and one report (3) has been noted of a study of the changes in female mice susceptible to spontaneous mammary carcinomas. In the latter, however, only the total P^{32} uptake was measured; no fractionation of the various P-containing constituents was attempted.

The responsiveness of the adrenal P^{32} turnover rates to pituitary stimulation indicates that this is a measure of adrenal cortical activity. Relatively small amounts of

P are found in the medulla and large changes would be necessary to produce detectable changes in the total P of the gland. Gemzell (24) found that the medulla accounted for only 25% of all the P in the adrenal gland and only 10% of all the labelled P in the organ was found there. Thus, although the cortex was not removed and studied separately, changes in the whole gland would undoubtedly be due to cortical changes. Riedel (84) has made extensive studies of the changes produced in the adrenal glands of the rat following hypophysectomy and ACTH administration and found a decreased incorporation of P into all of the fractions studied (the same fractions as used in the present investigation) following hypophysectomy. Furthermore, the levels tended to return towards normal upon administration of ACTH to the hypophysectomized animals.

Albert and Johnson (3) studied the total P^{32} uptake in the adrenals of low cancer (C57 black) and high cancer (DBA) mice. They also studied DBA mice in which the milk agent was absent. They found a decreased uptake in the presence of the milk agent and an increased uptake in pregnant mice of the DBA strain. The latter change is probably due to the stress effect of pregnancy.

If the tumor acts as a stress agent to stimulate the production of adrenal cortical hormones, it would be expected to produce changes in the adrenal gland similar to those produced by any general stress agent. Although no previous studies have been made of the effect of tumors on

adrenal P^{32} uptake, Reiss and Halkerston (83) and Nicholls and Rossiter (76) studied the effects of cold stress.

Reiss and Halkerston (83) measured the total radioactivity of the acid soluble P and found an increase of about 100 percent in animals kept in the cold for 2 hours over those at room temperature. Similar increases were found in animals treated with corticotrophic hormone and immature rats when taken from the mother and kept at room temperature. The effect was cumulative with young rats taken from the mother and placed in the cold. They also noted a reduction in P^{32} uptake in hypophysectomized rats which could be corrected towards normal by injection of corticotrophic hormone. They conclude that the changes are initiated by endogenous mobilization of corticotrophic hormone.

Nicholls and Rossiter (76) made similar cold-stress studies on the P^{32} uptake in the adrenals. The P-containing fractions were studied separately: inorganic P, 20-minute hydrolysable P, total acid soluble P. They also measured the uptake in the inorganic P of the plasma. With short periods of stress they found an increase in the relative specific activity of all of the adrenal fractions, with no change in the activity of the inorganic P of the plasma. With longer periods of stress the adrenal uptake remained elevated above normal and the plasma inorganic P uptake fell.

That there is a definite correlation between the pituitary action on the adrenal cortex and the rates of phos-

phorylation within the gland appears likely. A similar correlation between phosphorylation and adrenal cortical hormone production can only be surmised due to the inability of present analytical methods to measure circulating hormone levels. Such a correlation, however, is indicated.

E. METABOLISM STUDIES OF THE LIVER

The liver is very susceptible to changes in the general condition of the body. This is shown particularly well in the case of the tumor-bearing host, where there is a great upset in a large number of liver functions. A number of enzyme activities are altered, there is a change in the concentration of some constituents, and an increase in the size of the organ.

Turnover studies in the livers of tumor-bearing animals have centred primarily on the nucleic acids. It has been noted that there is an increased incorporation of P^{32} into the liver desoxyribose nucleic acid (DNA) of tumor-bearing hosts (55, 56, 78, 109). The ribonucleic acid (RNA) has a much higher rate of P^{32} incorporation than that of DNA, but does not change in the tumor animal (16, 78). This would indicate that the acceleration occurs somewhat late in the synthesis of the DNA, since an increased rate of incorporation in a precursor common to both DNA and RNA would be expected to raise the RNA specific activity as well as that of DNA.

Payne, Kelly, and Jones (77) labelled different por-

tions of the nucleic acids in normal mice and concluded that there was not an independent turnover of any one portion of the nucleic acid molecule. This would indicate that new molecules must be entirely elaborated from the precursors, rather than from replacement of portions of previously-formed molecules.

It has been shown (39) that P^{32} enters the DNA only at the time of mitosis and it may be concluded (16) that DNA is extremely stable in the non-growing organ.

The increase in DNA specific activity is proportional to the size of the tumor. It has been suggested (56) that the increase may be due to a metabolic effect following an increased demand for tissue synthesis or a specific humoral agent elaborated by rapidly dividing tissues. The increased P^{32} uptake in liver DNA is also found in association with pregnancy (4, 56).

The increased liver weight in tumor-bearing animals is due to a true increase in the number of cells, although there is some increase in water content (65). The ratio of the liver weight to the tumor weight in rats and mice remains constant (5). Thus it is conceivable that the increased mitotic activity of the liver is responsible for the increased DNA activity. It has been shown (4) that the high DNA uptake of P^{32} in spontaneous mammary tumors in mice is not due to an increase in the DNA concentration per cell, but to an increased cellularity.

F. METABOLISM STUDIES OF THE TUMOR

The Walker 256 carcinoma is, like others of its type, a rapidly growing tumor and, as such, must obtain larger amounts of energy than normal resting tissues. The great increase in size of the tumor, with its concomitant synthesis of building blocks, cannot take place without the expenditure of considerable energy. Although the nature of the degradation of raw materials in the tumor tissue has been studied for many years by numerous investigators, no new pathways of metabolism have yet been found. It is possible that, in the future, some new specific enzyme or system of enzymes will be found in tumor tissue or that some enzymes found in normal tissues will be shown to be specifically lacking in tumor tissue. Nevertheless, all of the differences noted to date are of an entirely quantitative nature.

The accumulation in tumors of lactic acid, an alternative to the citric acid cycle, originally led workers to believe that the latter was not functioning in tumor cells. Later work has shown, however, that all of the necessary components are present (111) and that the system functions (112). This still leaves unexplained the accumulation of lactate. Warburg (110) noted that there was an almost equal oxygen consumption in normal and neoplastic tissue. The citric acid cycle which removes the lactate is functioning at a normal

THE HISTORY OF THE

REIGN OF THE

EMPEROR

OF THE

CHINESE

EMPEROR

OF THE

CHINESE

EMPEROR

OF THE

CHINESE

EMPEROR

OF THE

CHINESE

EMPEROR

OF THE

CHINESE

EMPEROR

OF THE

CHINESE

EMPEROR

OF THE

CHINESE

EMPEROR

OF THE

CHINESE

EMPEROR

OF THE

CHINESE

EMPEROR

rate; however, glycolysis, the anaerobic portion of the degradation of metabolites, may be functioning at a greater rate and producing more lactate than can be handled by the subsequent stages.

Analysis (58) has shown the intermediates of the glycolytic (Embden-Meyerhoff) pathway to be present in tumor tissue in quantities similar to those in normal tissue. That this system functions and produces lactate has been shown (19, 53). The predominance of this anaerobic pathway in tumor tissue is similar to that found in lower types of animal life (e.g. yeast) and may indicate a reversion, on the part of the tumor cell, to a more primitive type of existence.

The glycolytic pathway is essentially one of phosphorylation (58) involving the use and formation of high energy phosphate bonds. A net increase in bonds furnishes some of the energy necessary for other functions of the tissue. "The synthesis of high energy phosphate bonds during glycolysis and during certain types of aerobic oxidations probably provides much of the energy required for cell maintenance and growth." (30).

The importance of phosphorylation to the tumor cell is shown by the fact that tumor tissue will take up and retain P^{32} for a longer period of time than will normal tissue (52). Adenosine diphosphate and triphosphate, accessories to the phosphorylation mechanism, have been

shown to have a rapid in vivo turnover in tumor tissue (58). The rate of renewal of phospholipid P is also high, being near the values for the most active normal tissues (39).

METHODS

A. ANIMALS

All of the animals used were male albino rats of the Wistar strain, obtained locally and from St. Louis, Mo. The Walker 256 carcinoma was grown in the muscles of both hind legs of each rat.

Carrier rats, not used in the experiments, were sacrificed and the tumors, usually grown subcutaneously in the back, were aseptically removed. A portion of non-necrotic tissue from the tumor was homogenized in normal saline using an all-glass homogenizer of the Potter-Elvehjem type (81). Care was taken not to homogenize too thoroughly, since it has been shown that whole cells are necessary for the tumor to grow (108). 0.2 mls of the tumor cell suspension produced was injected into the leg muscles using a syringe fitted with a large bore needle. Photographs of the tumorous animal and the excised tumor are shown in Figs. 2 and 3.

The animals were kept in large cages for freedom of movement and received food and water ad libitum until 24 hours before sacrifice, at which time the food was removed.

Control animals of approximately the same age and



Fig. 2. The Walker 256 carcinoma after 20 days of intramuscular tumor growth.

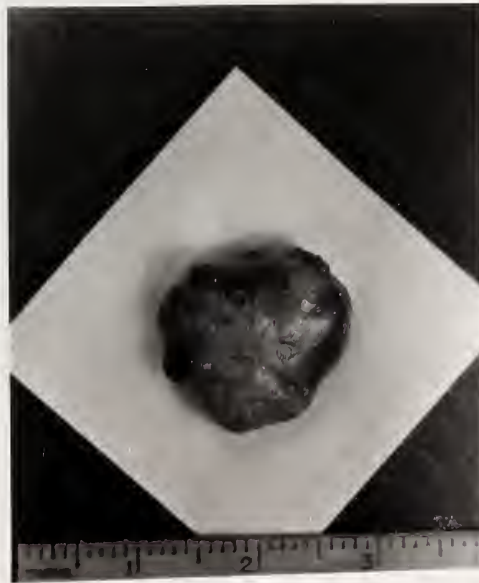


Fig. 3. Excised Walker 256 carcinoma after 20 days of intramuscular tumor growth.

weight were carried along with each group of tumor animals to indicate any small sources of possible variation and to serve as an indication of normal conditions. There was some variation between different groups of tumor animals under the same conditions of tumor age. This variation contributed to the high standard errors noted in some cases. Gemzell (83) noticed an extreme variation in P^{32} uptake of the adrenal cortex and concluded that it was due to storage of the animals in different rooms. Mider (67) has commented that only about 80% of tumor animals are characteristic, the other 20% show extreme variations.

B. INJECTIONS

All animals received an intraperitoneal injection of 200 microcuries (μc) of P^{32} regardless of weight. It has been shown (14, 57) that a dose much larger than 200 μc is necessary to have any effect on the metabolism of the animal. Since the use of the same dose in each animal would tend to produce variations in the activities in animals of different weights, owing to different dilution factors, the plasma activity figures were multiplied by the animal weights. The tumor blood supply is continuous with that of the host, so the total weight, rather than the carcass weight, was used. Multiplication is unnecessary in the case of the tissue fraction relative specific activities, as all values are expressed relative to the plasma specific activity of that animal.

The animals in the hormone studies received a single intraperitoneal injection of 4 mgm. of ACTH (Acton, Nordic) or 10 mgm. of Cortisone (Cortone, Merck), administered 4 hours prior to P³² injection (12 hours prior to sacrifice). Riedel (84) has shown that it is necessary to give ACTH before P³² in order to detect an altered P³² incorporation in hypophysectomized rats.

C. TISSUES

The animals were killed at varying intervals after administration of P³². Under ether anaesthesia, a vertical incision was made up the abdomen. A blood sample was withdrawn from the aorta by means of a heparinized syringe. It was centrifuged in the cold room as soon as convenient.

The adrenals were removed, cleared of adhering tissue, and plunged immediately into liquid nitrogen to arrest any further metabolic processes and the breakdown of the less stable constituents.

A sample of the central lobe of the liver weighing approximately 200 mgm. was removed, blotted, and transferred to the liquid nitrogen. A portion of the actively growing tumor tissue was removed and treated similarly.

The tissues were kept in the frozen state while being weighed and until homogenized.

D. SEPARATION OF THE P COMPOUNDS

The chemical separation of the P compounds is based upon the procedure reported by Schneider (92). A more

detailed account of the exact procedure used will be found in Appendix A.

All of the homogenizations and acid soluble fraction separations were carried out in a cold room maintained at 3°C. to eliminate, as much as possible, any changes in the various fractions. Determinations of the inorganic P of blood, adrenals, liver, and tumor were also carried out in the cold.

The adrenal, liver, and tumor samples were treated in the same manner except for differences in quantities and in the number of washes used.

The general separation scheme is indicated in Fig. 4.

1. Plasma Inorganic P

200 microlitres of plasma were removed from the centrifuged blood sample and extracted 3 times in the cold with 10% trichloroacetic acid (TCA). An aliquot of the combined liquids was taken for the P estimation.

2. Acid Soluble P

The tissues were homogenized in 10% TCA in a Potter-Elvehjem homogenizer (81) and transferred to a centrifuge tube. The residue was washed in the cold with additional portions of 10% TCA and the supernatants combined. Aliquots of this fraction were then taken for Inorganic P, ATP P, and Total Acid Soluble P measurements.

3. Inorganic P

While still in the cold room, an aliquot was immediately taken for the inorganic P determination. Speed and cold are

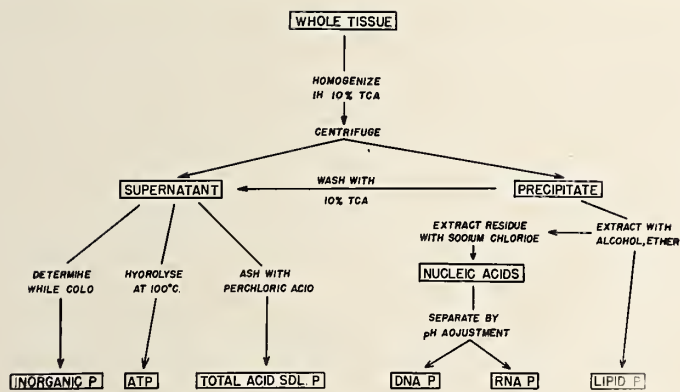


Fig. 4. General separation scheme for the P-containing compounds.

essential, since any creatine phosphate would soon break down at higher temperatures, increasing the value for the inorganic P. Riedel (84) has shown that the creatine P of liver does not break down under the conditions used, and has also shown that there is no detectable creatine P in the adrenal gland.

4. Adenosine Triphosphate (ATP) P

An aliquot from the acid soluble P fraction was hydrolysed for 20 minutes with 60% perchloric acid. This procedure releases the two labile phosphates and an inorganic P determination will then indicate the total value for inorganic P plus that released from ATP. By subtracting the previously-determined inorganic P, the portion due to ATP can be determined. In the case of the liver samples this value is unavoidably increased by the presence of creatine P which breaks down under these conditions.

5. Total Acid Soluble P

Ashing of an aliquot from the acid soluble fraction for 10 minutes with 60% perchloric acid served to release all of the P in the fraction. The additional P is due to the third phosphate of ATP, hexose phosphates, etc.

6. Lipid P

The residue from the acid soluble extraction was treated with cold alcohol, then a boiling alcohol-ether mixture, and finally with ether at room temperature. A portion of the combined extracts was taken and ashed with

perchloric acid before a P estimation.

7. Nucleic Acid P

The residue from the lipid P extractions was further extracted with 10% sodium chloride at 100°C. according to the method of Hurlbert and Potter (44). The extracts were combined and the nucleic acids precipitated by the addition of 95% alcohol and storage overnight at 3°C. After washing with alcohol, the precipitate was dissolved and hydrolysed in 0.1N sodium hydroxide for 18 hours at 37°C. The addition of cold concentrated HCl to the chilled NaOH extract served to precipitate the DNA, according to the method of Tyner et al (109). The RNA remained in the supernatant which was removed for P estimation.

After dissolving in NaOH a P estimation was carried out on the DNA fraction.

E. PHOSPHORUS ESTIMATION

P was estimated by the method of Ernster et al (22). The P was converted to phosphomolybdate with perchloric acid and ammonium molybdate after which it was shaken with a 1:1 mixture of isobutanol and benzene. The P passed quantitatively into the organic layer, an aliquot of which was removed and treated with a solution of stannous chloride. The blue color produced was read in a Beckmann Model B spectrophotometer at 730 mμ. As shown in Fig. 5, Beer's law was satisfied over the range investigated.

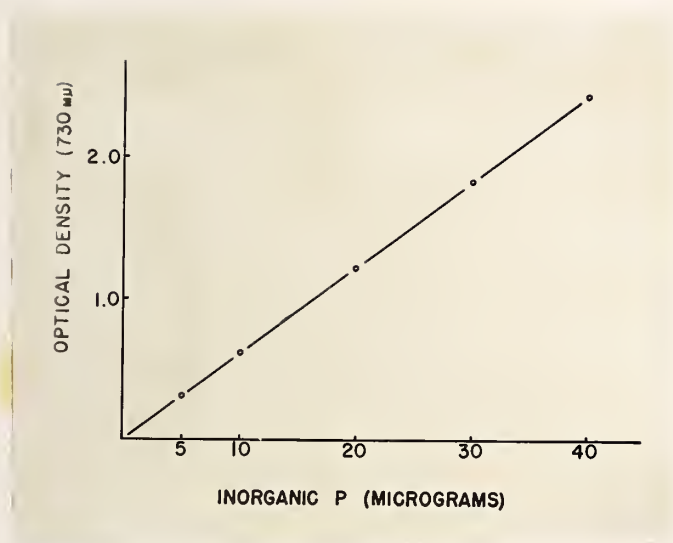


Fig. 5. The relationship between optical density at 730 mμ and concentration of P.

F. RADIOACTIVITY DETERMINATION

The radioactivity of the sample was measured by placing the blue solution from the P estimation directly into an M-6 liquid Geiger-Müller counter tube (20th Century Electronics). The counts were recorded by a Super-scaler (Tracerlab, Inc., Boston, Mass.). The background counting rate was subtracted to give the radioactivity due to the P^{32} in the sample which was then corrected for decay. Counting was continued for 3 minutes. In the case of particularly active samples (over 800 counts per minute) counting was for 1 minute with no loss of accuracy (82).

G. DEFINITION OF TERMS

Specific Activity (S/A) of a sample refers to the number of counts per minute divided by the number of micrograms of total P in that sample. Thus it is an indication of the concentration of P^{32} in that sample.

Relative Specific Activity (Rel. S/A) refers to the specific activity of a sample expressed as a percentage of the specific activity of the plasma inorganic P for that same animal.

Corrected Specific Activity (Corr. S/A) is used to correct the specific activity of the plasma inorganic P for differences in dilution (due to different animal weights) in the various animals, since a constant dose of P^{32} was given. It refers to the plasma inorganic P Spec-

ific activity multiplied by the weight of the animal times 10^{-3} . The adrenal and liver relative specific activities need no correction.

H. STATISTICAL ANALYSIS

All groups of values were analyzed statistically and the standard error of the mean (S.E.M.) determined. The averages for the tumor animals were compared with the corresponding values for normal animals by use of the Fisher t test:

$$t = \frac{m_1 - m_2}{\sqrt{s_1^2 + s_2^2}}$$

where m = mean
 s = standard error
of the mean

The probability (p) of a similar difference due to chance was determined from the t value by the use of Fisher's table of t . A probability less than 0.05 ($p < .05$) is considered "significant", a value lower than 0.01 ($p < .01$) is "highly significant." In the presentation of results these terms will apply. Where no mention is made of significance it may be assumed that the difference is not statistically significant ($p > .05$).

I. AUTORADIOGRAPHY

The adrenals were removed from a rat which had been injected 6 hours previously with 200 microcuries of P^{32} . Holt et al (41) have shown that most of the P^{32} is present in tissue in a water-soluble form and will be removed by ordinary methods of histological preparation. They recommended a freezing and vacuum dehydration technique.

After freezing in solid carbon dioxide the adrenals were placed in a test tube and the air evacuated to 0.05 mm Hg. A jacket containing a freezing mixture of CaCl_2 and ice placed around the tube kept the adrenals frozen while the water was being removed. After 24 hours the adrenals were found to be dry and were imbedded in paraffin. 8 micron slices were taken and placed on glass microscope slides. The paraffin was removed with xylol. Holt and Warren (40) have shown that xylol does not extract detectable amounts of P^{32} from tissues. Furthermore, they also found that small traces of paraffin remaining did not scatter the radiation from the tissue.

Sections of X-ray film were fastened against the tissue slices for 16 days. A piece of cellophane was used between the tissue and the film to prevent any chemical action on the emulsion. The film was developed in the usual manner.

RESULTS

A. THE EFFECT OF TIME AFTER P^{32}

A study of the effect of time after P^{32} injection is necessitated by the fact that some of the more active fractions will respond quickly to changes in the isotope concentration of their precursors and will thus reach equilibrium with them quickly. A comparison of a certain fraction from an experimental animal with that from a control animal may show both to have relative specific activities near 100 at a long interval after P^{32} . The fact that the fraction from one animal took longer to reach that equilibrium would not be indicated. This could only be shown by study at a time interval when the fraction is approaching, but has not attained, equilibrium.

Similarly, the more slowly metabolizing fractions will incorporate P^{32} slowly and reach equilibrium only after a long time interval. If studied a short time after P^{32} the difference between the two animals may be relatively high but absolutely low, since all of the values will be low. In addition, low specific activities are the result of a low number of counts per minute where the accuracy of the experimental procedure is not as great as at a high counting rate.

Since the relative specific activities are different for the different fractions within one tissue and also different for the same fraction in different tissues, it is necessary to cover a wide range of time intervals after P^{32} . In this investigation the fractions were studied at 2, 4, 8, and 16 hours after injection of the P^{32} . Inorganic P, ATP P, and total acid soluble P were found to attain equilibrium rapidly, lipid P slowly, and nucleic acid P very slowly. In the case of tumor animals, an interval of 15 days after inoculation of the tumor cells was chosen as it seemed to represent the period of most rapid growth of the tumor. Any changes might be expected to be the greatest at this interval.

The relative specific activities for the ATP P are derived by calculation from the inorganic P and the fraction obtained upon hydrolysis of the acid soluble P for 20 minutes. From the counts per minute and P concentrations of the latter are subtracted the respective values for the inorganic P. Further calculation yields the relative specific activities. Thus the fraction represented is the esterified P which can be hydrolysed to an inorganic form in 20 minutes at 100°C . This may be more properly called the 20-minute-hydrolysable acid-soluble P. It contains the 2 labile phosphates from ATP and, in the case of liver samples, will also include P derived from creatine phosphate. It will probably also contain the labile phosphate from ADP and may include other constituents.

Since it was obtained by calculation from two fractions, any experimental errors in the ATP P would be sum of the errors in the other two fractions. Calculation showed the standard errors of the mean to be high and the averages variable. Therefore, because of the variability of the values and the ambiguity of the fraction being studied, the ATP P has been omitted from further discussion.

1. Plasma

The corrected specific activities of the plasma inorganic P for the tumor animals followed very closely those for normal animals (Fig. 6). Gemzell (24) found that the inorganic P of the plasma in normal rats increased rapidly to a maximum in $7\frac{1}{2}$ to 10 minutes. The P^{32} concentration in the plasma then dropped rapidly due to diffusion into the extracellular fluid and exchange with the large P reservoir in the bones. The increased specific activity found in hypophysectomized animals (25, 84) was absent. Gemzell and Samuels (25) have shown that the level of the P in the plasma may be controlled by growth hormone.

Nicholls and Rossiter (76) found no change in the specific activity of the plasma inorganic P following short periods of cold stress and an increase with long periods of stress.

The values obtained in this investigation followed closely those noted by Riedel (84) in normal animals.

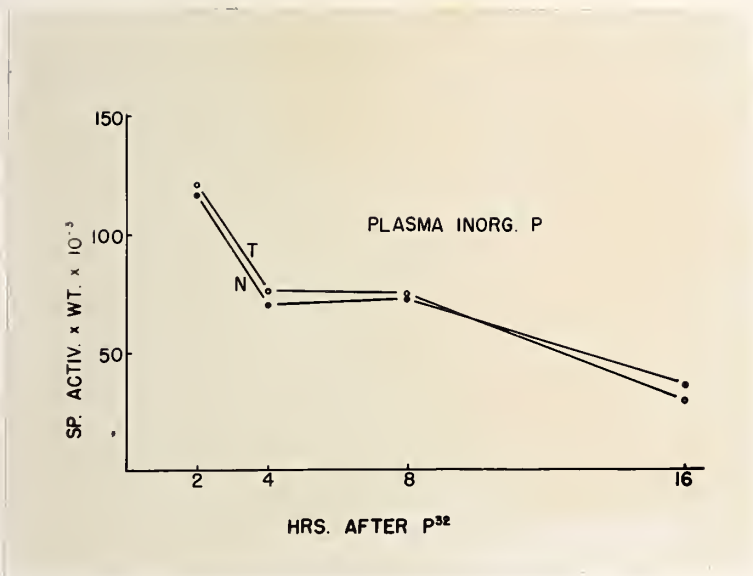


Fig. 6. The change in the corrected specific activity of the inorganic P of the plasma with time after the administration of P³² for normal (N) and tumor-bearing (T) animals.

2. Adrenals

(a) Inorganic P

The relative specific activity of the inorganic P of the adrenals was depressed in the tumor animals at both 2 and 4 hours after P^{32} administration (Fig. 7). The latter change was highly significant ($p < .01$). The rise beyond 100 at 4 hours, the drop at 8 hours, and then the slight rise again has been previously noted in normal animals (84) and is due to the more rapid decrease in the specific activity of the inorganic P of the plasma than that of the adrenal. A similar situation was not found in the case of the tumor animals.

By the 8 hour interval equilibrium with the plasma inorganic P had been reached and there was no longer a difference between normal and tumor animals. The difference noted at 16 hours was not statistically significant.

Hypophysectomy, on the other hand, has been shown to decrease the relative specific activities of the adrenal inorganic P at all time intervals (25, 84).

(b) Total Acid Soluble P

The changes in the relative specific activity in both normal and tumor animals (Fig. 8) followed very closely those found in the inorganic P of the adrenal. The difference at 4 hours, although not quite as great, was again highly significant.

Equilibrium had been reached by 8 hours and the dif-

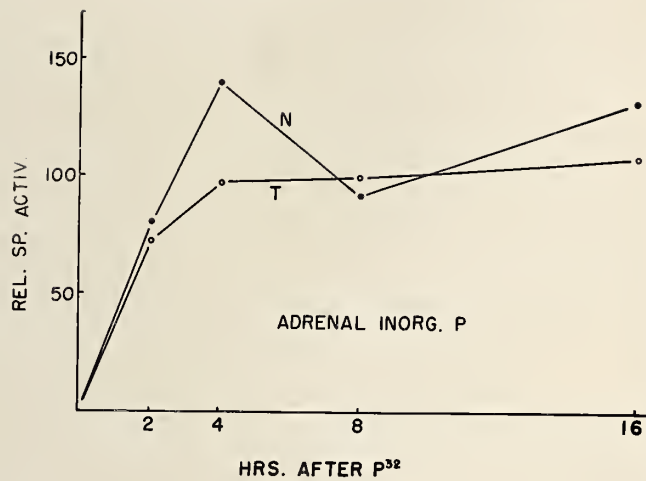


Fig. 7. The change in the relative specific activity of the inorganic P of the adrenal with time after administration of P^{32} for normal (N) and tumor-bearing (T) animals.

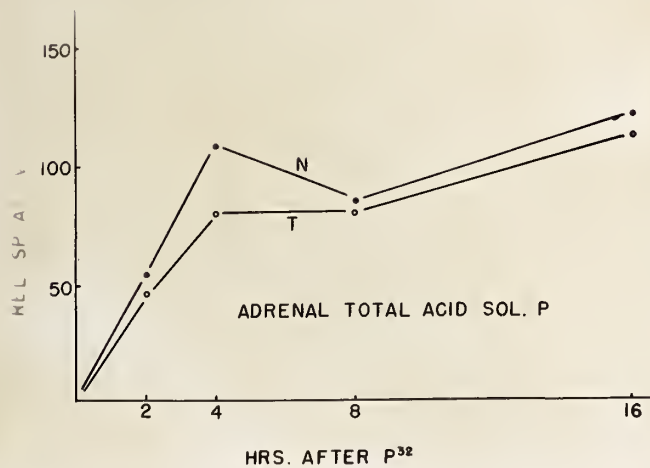


Fig. 8. The change in the relative specific activity of the total acid soluble P of the adrenal with time after administration of P^{32} for normal (N) and tumor-bearing (T) animals.

ference between tumor and normal animals had vanished, as was the case with the inorganic P.

(c) Lipid P

The lipid fraction of the adrenals incorporated P^{32} slowly (Fig. 9) and equilibrium had not yet been reached by 16 hours, the longest time interval studied. The value in tumor animals was about two thirds of normal at the 2 hour interval and half at 4 hours. The former difference was not statistically significant due to the high standard error of the mean associated with such low counts. The difference at 4 hours was highly significant. The absence of a difference at 8 and 16 hours might be explained by the fact that the inorganic P, the ultimate precursor of the lipid P, had attained equilibrium and its relative specific activity was equal to that found in normal animals. This increased P^{32} concentration in the precursor raised the concentration in the product.

(d) RNA P

P^{32} was incorporated slowly into the RNA (Fig. 10); the relative specific activity was only about thirty at 16 hours after administration of the P^{32} . None of the differences between tumor-bearing and normal animals were statistically significant.

(e) DNA P

In view of the changes found in the DNA P of the liver

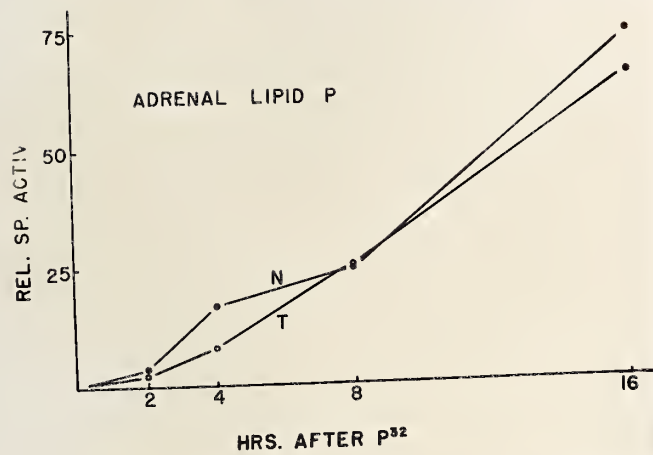


Fig. 9. The change in the relative specific activity of the lipid P of the adrenal with time after administration of P^{32} for normal (N) and tumor-bearing (T) animals.

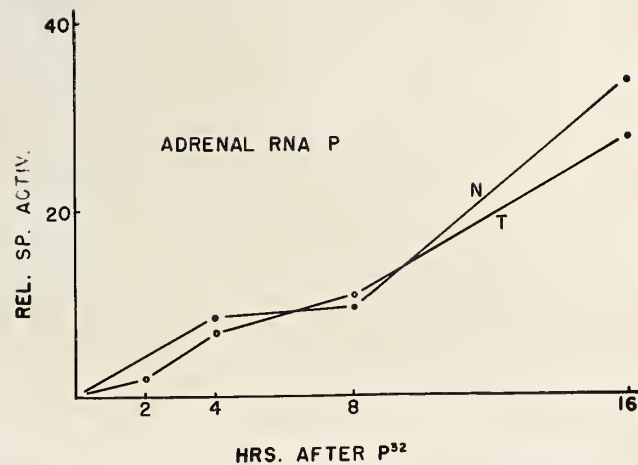


Fig. 10. The change in the relative specific activity of the RNA P of the adrenal with time after administration of P^{32} for normal (N) and tumor-bearing (T) animals.

it is unfortunate that determinations could not be made in the case of the adrenals. The great increase found in the relative specific activity in tumor animals may be a result of the increase in liver size. It would be interesting, therefore, to see whether the adrenals, which are undergoing a rapid hypertrophy, show a similar increase.

The incorporation of P^{32} into the DNA of tissues takes place very slowly, as will be seen in the case of the normal livers, and the counting rate in the case of a sample as small as the adrenals is barely above background count. The inability to measure the adrenal DNA P specific activity could be corrected by pooling a number of adrenals or by increasing the initial dose of P^{32} to the animal. Both of these were inadvisable in the present investigation.

The adrenal DNA P activities would bear investigation by one of the above methods.

3. Liver

(a) Inorganic P

The inorganic P of the liver reached equilibrium with the plasma inorganic P very rapidly (Fig. 11); the relative specific activity was above 100 by 2 hours. This is somewhat more rapid than in the case of the adrenals, where the relative specific activity was approximately 75 at the 2 hour interval. The values for tumor and normal animals were quite close at 2, 4, and 8 hours after P^{32} . The decreased relative specific activity in tumor animals at 16

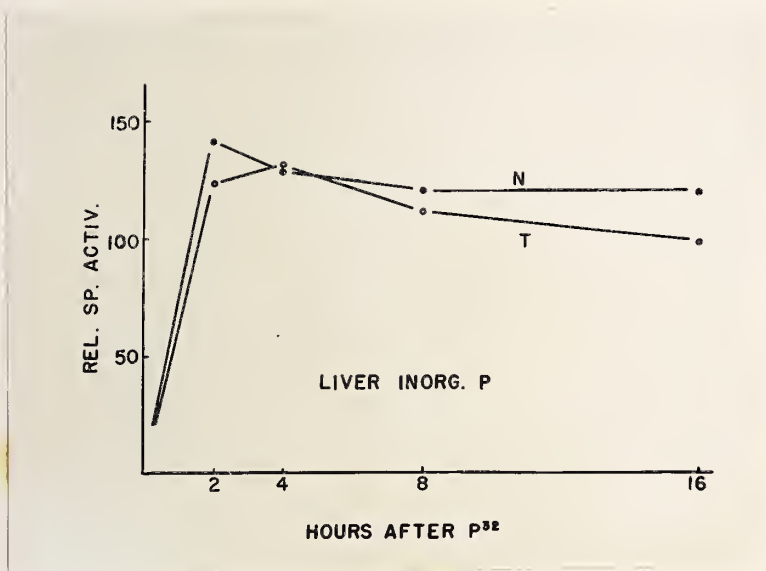


Fig. 11. The change in the relative specific activity of the inorganic P of the liver with time after administration of P^{32} for normal (N) and tumor-bearing (T) animals.

hours is statistically significant. This is difficult to explain since the inorganic P has been at equilibrium with the plasma inorganic P for at least 14 hours. A depressed rate of incorporation is probably not responsible, since this would show itself at an earlier time interval. The specific activity of the plasma inorganic P was low at 16 hours and P^{32} was passing from the intracellular to the extracellular space. Thus it could be postulated that there is, in tumor animals, an accelerated removal of P^{32} from the inorganic P of the tissue to that of the plasma. The significance of this is obscure at present.

(b) Total Acid Soluble P

Equilibrium was again reached by 2 hours (Fig. 12) as compared to more than 4 hours in the case of the adrenal total acid soluble P. The relative specific activities for normal and tumor animals were very close except at the 16 hour interval where the difference was again significant. The change here was probably due to a similar difference in the inorganic P which forms part of the total acid soluble P and is the precursor for the remainder.

(c) Lipid P

The rise in relative specific activity in normal animals was gradual and reached 100 by 16 hours after administration of P^{32} (Fig 13). This was more rapid than in the case of the adrenals where the relative specific activity of the lipid P was only about 70 in normal animals

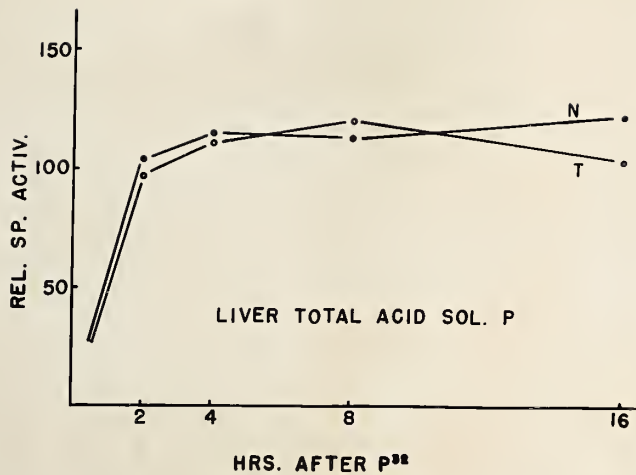


Fig. 12. The change in the relative specific activity of the total acid soluble P of the liver with time after administration of P³² for normal (N) and tumor-bearing (T) animals.

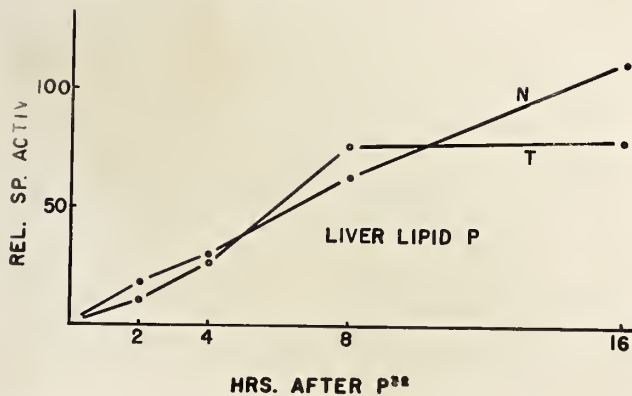


Fig. 13. The change in the relative specific activity of the lipid P of the liver with time after administration of P³² for normal (N) and tumor-bearing (T) animals.

at the same time interval.

The significant difference at 16 hours may be attributed to a similar change in the inorganic P and total acid soluble P. The increased relative specific activity in tumor animals at 8 hours was small but also highly significant.

(d) RNA P

The rise in relative specific activity in normal animals was gradual and slightly slower than that in the adrenals of normal animals (Fig. 14). This is in contrast to the fact that the inorganic P, total acid soluble P, and lipid P attain equilibrium more rapidly in the liver than in the adrenals.

The highly significant increase in relative specific activity in tumor animals 8 hours after P^{32} cannot be explained in the same manner as the increase noted in the relative specific activity of the adrenal inorganic P and total acid soluble P, since the difference had vanished at the 16 hour interval although equilibrium had not yet been attained. The change may be related to the great change in liver DNA activities in tumor animals. Another explanation is that the liver RNA relative specific activity was affected by that of the tumor tissue where a similarly high value at 8 hours and low value at 16 hours was noted (Fig. 19).

Greenstein (30) has commented that the tumor attempts to impose its own metabolism on that of the host. The

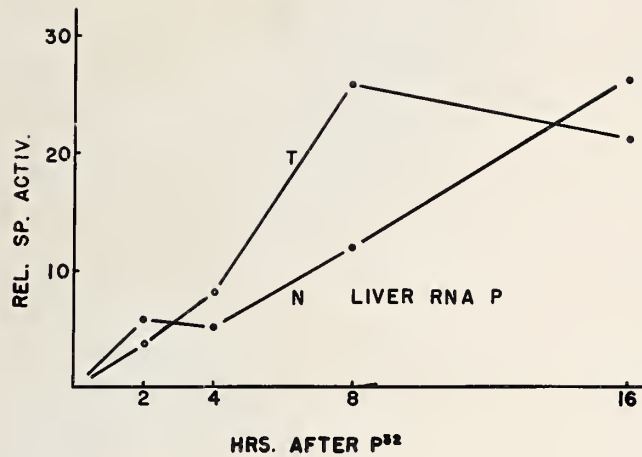


Fig. 14. The change in the relative specific activity of the RNA P of the liver with time after administration of P³² for normal (N) and tumor-bearing (T) animals.

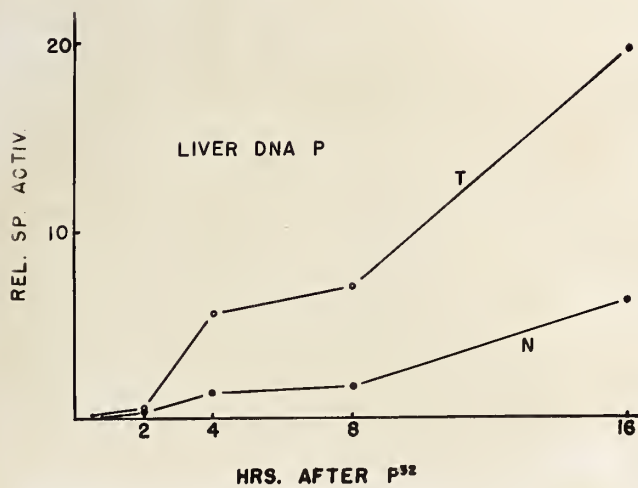


Fig. 15. The change in the relative specific activity of the DNA P of the liver with time after administration of P³² for normal (N) and tumor-bearing (T) animals.

similarity of the changes in relative specific activity of the liver and tumor RNA would support this statement. The acid soluble and lipid fractions would not show this alteration since the curves are already quite similar to the corresponding curves for normal animals. The DNA relative specific activity of the liver was affected by another factor.

(e) DNA P

The greatly increased relative specific activity in tumor animals was significant at 4 hours and highly significant at 8 and 16 hours after P^{32} (Fig. 15). The values at two hours were so low as to increase the standard errors sufficiently to destroy significance.

The increased DNA relative specific activity has been noted in previous work (56, 78, 109) and is commented upon in the historical background. The high activity has been related to mitotic activity (39) and previous work with the Walker 256 carcinoma has shown an increase in the size of the liver (5). The liver weights were not measured in the present investigation, hence a correlation between growth and DNA P^{32} incorporation cannot be made. The relative specific activities in the tumor animals were 3 to 4 times those in normal animals and, if it is assumed that the incorporation of P^{32} into DNA is a direct measure of mitosis, it may be postulated that new cells are being formed at 3 to 4 times the normal rate.

3. Tumor

(a) Inorganic P

The inorganic P of the tumor tissue reached equilibrium rapidly (Fig 16), although not as fast as was the case with the liver. One of the reasons for selecting the liver for study was that it is a rapidly metabolizing tissue and would form a basis for comparison with the relative specific activities of the tumor tissue.

(b) Total Acid Soluble P

The relative specific activity of the total acid soluble P followed very closely the relative specific activity for the inorganic P, being only slightly lower at 2 and 4 hours, and higher at 8 and 16 hours (Fig. 17). The relative specific activity of the total acid soluble P in the case of the liver and adrenal samples was considerably lower than that of the inorganic P of the respective tissues. It is probable that the portion of the total acid soluble P which is not inorganic P itself is formed from inorganic P by a mechanism of phosphorylation. The close proximity of the total acid soluble P relative specific activity curve to that of the inorganic P curve would indicate a high rate of phosphorylation in the tumor tissue.

(c) Lipid P

The relative specific activity of the lipid P rose slowly in tumor tissue and had not reached equilibrium by 16 hours (Fig. 18), whereas the lipid P of the liver of

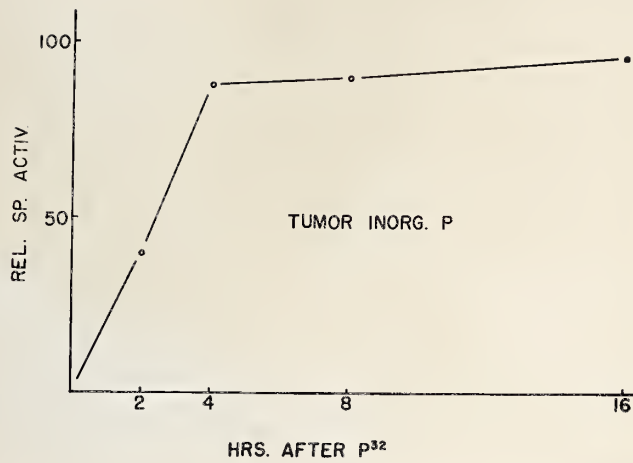


Fig. 16. The change in the relative specific activity of the inorganic P of the tumor with time after administration of P³².

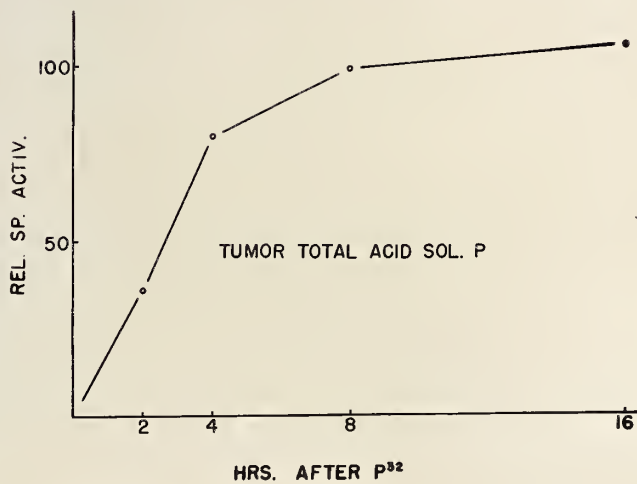


Fig. 17. The change in the relative specific activity of the total acid soluble P of the tumor with time after administration of P³².

normal animals had a relative specific activity over 100 at the same time interval and was almost 100 in the case of tumor animals. Thus the high rate of phosphorylation postulated in the tumor cell is not evident in the case of lipid P.

(d) RNA P

The relative specific activity of the RNA P rose rapidly up to 8 hours; the values were more than double those for normal liver tissue (Fig. 19). There was then a sudden drop to a low relative specific activity at 16 hours. The reason for this fall in the relative specific activity cannot be explained but may be related to the altered metabolism or the fast growth of the tissue. Whatever its reason, it appears to have had a profound effect on the relative specific activity of the RNA P of the liver where a similar sharp rise to a high value at 8 hours, then a drop at 16 hours, was noted (Fig. 14).

(e) DNA P

The relative specific activity of the tumor DNA P rose rapidly until the 8 hour interval (Fig. 20), an effect which may be associated with the accelerated rate of mitosis in the rapidly growing tumor tissue. However, the level ceased to rise after 8 hours, whereas it continued to rise in the case of the liver DNA P of tumor animals. This levelling of the curve may be associated with the drop in the relative specific activity of the tumor RNA P at

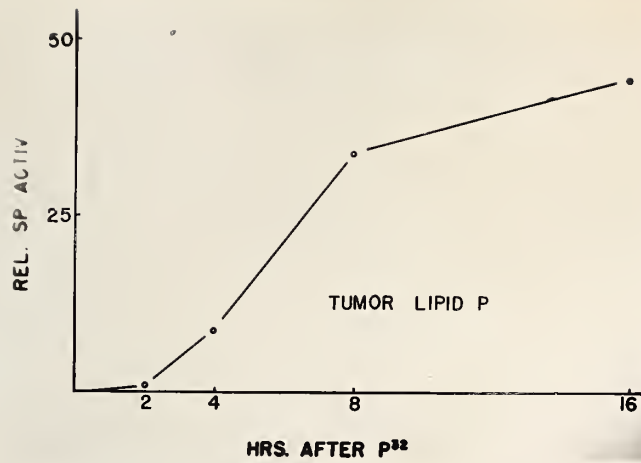


Fig. 18. The change in the relative specific activity of the lipid P of the tumor with time after administration of P^{32} .

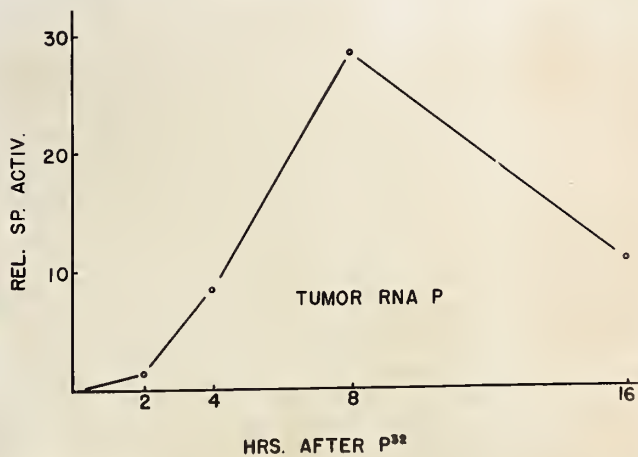


Fig. 19. The change in the relative specific activity of the RNA P of the tumor with time after administration of P^{32} .

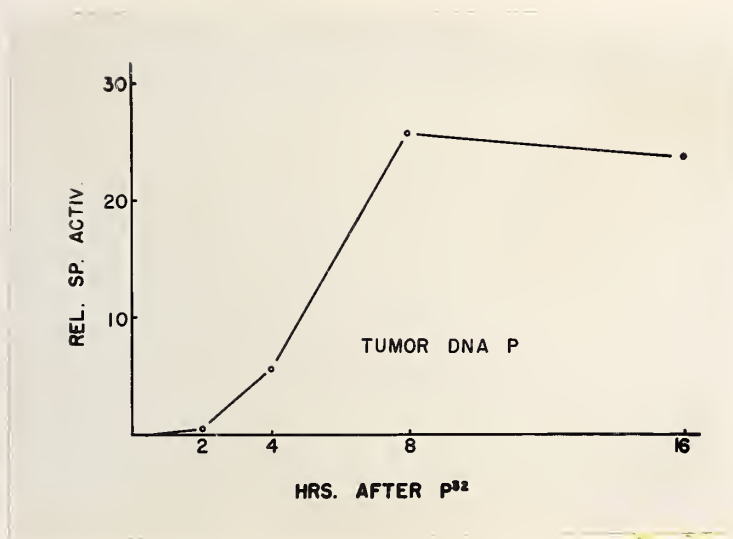


Fig. 20. The change in the relative specific activity of the DNA P of the tumor with time after administration of p^{32} .

16 hours.

B. THE EFFECT OF TIME AFTER TUMOR INNOCULATION

This study was initiated in an attempt to detect any changes in the relative specific activities of the various fractions as the tumor grew. In most cases the differences noted were small and, in the case of the fractions which had attained equilibrium before study (the 16 hour interval was selected), of dubious significance.

1. Plasma

There was a slight drop in the corrected specific activity of the plasma inorganic P 10 days after tumor inoculation (Fig. 21) as compared to normal animals. It was further depressed and highly significant at 15 days, the value after 20 days of tumor growth was back close to normal. The exact significance of this drop and then the recovery could not be explained but was also noticed in many of the other tissue fractions.

2. Adrenals

In all of the tissue fractions a drop in the relative specific activities at the 10 day interval was noted, followed by a recovery (Figs. 22, 23). The values for total acid soluble P were statistically significant and those for lipid P and RNA P highly significant. In all fractions except RNA P recovery was evident at 15 days. This is sooner than in the case of plasma and, as will be seen, many of the liver fractions.

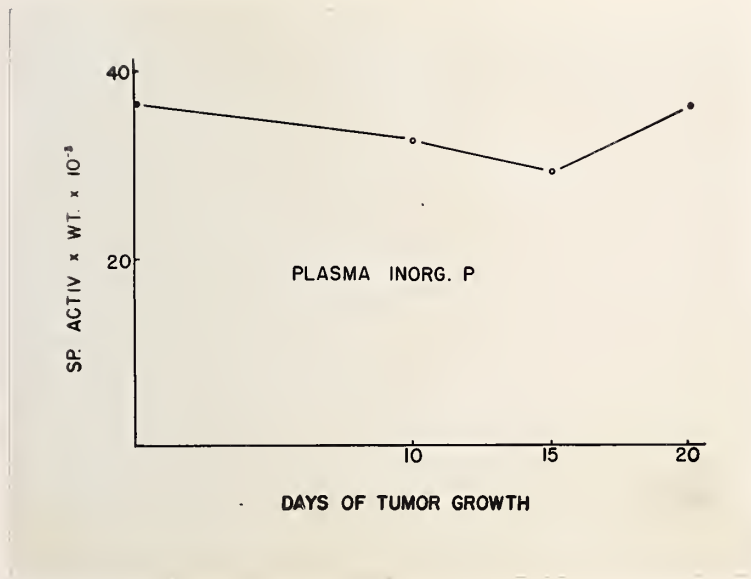


Fig. 21. The change in the corrected specific activity of the inorganic P of the plasma with time after tumor inoculation.

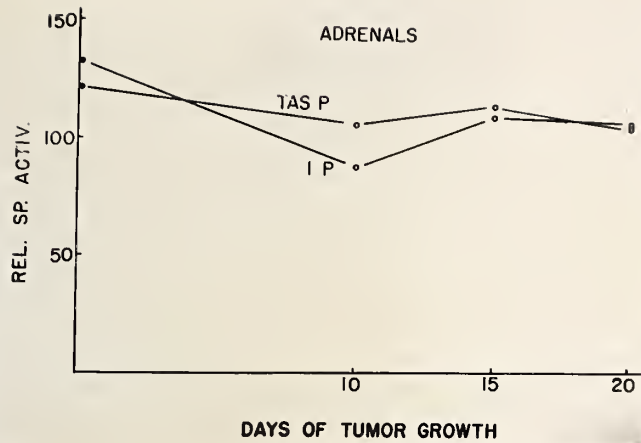


Fig. 22. The change in the relative specific activities of the inorganic P and total acid soluble P of the adrenals with time after tumor inoculation.

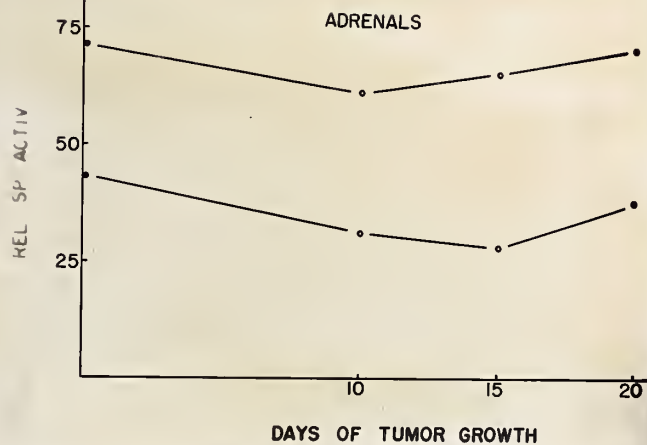


Fig. 23. The change in the relative specific activities of the lipid P (above) and RNA P (below) of the adrenals with time after tumor inoculation.

3. Liver

A slight drop in the relative specific activities of inorganic P, total acid soluble P, and lipid P at 10 days (Fig. 24) was increased by 15 days and then followed by a recovery almost to normal in the case of inorganic P and total acid soluble P and above normal in lipid P. The high relative specific activity of the lipid P may be associated with the extreme lipemia noted at this stage of the tumor growth.

The relative specific activity of the DNA P was greatly increased from normal at both the 15 (highly significant) and 20 day intervals (Fig. 25). No figures were obtained at 10 days after tumor implantation. The slight fall at 20 days may indicate that the liver has slowed its rate of hypertrophy. It has been shown that the liver begins to break down in the very late stages of tumor growth (96).

The great rise in the relative specific activity of the RNA P at 20 days after tumor inoculation cannot be explained.

4. Tumor

There are no statistically significant changes in the inorganic P or total acid soluble P relative specific activities as the tumor grows (Fig. 26). The lipid P and RNA P showed decreased relative specific activities at the 15 day interval. The changes were significant and were corrected in the 20 day tumor animals. The high relative

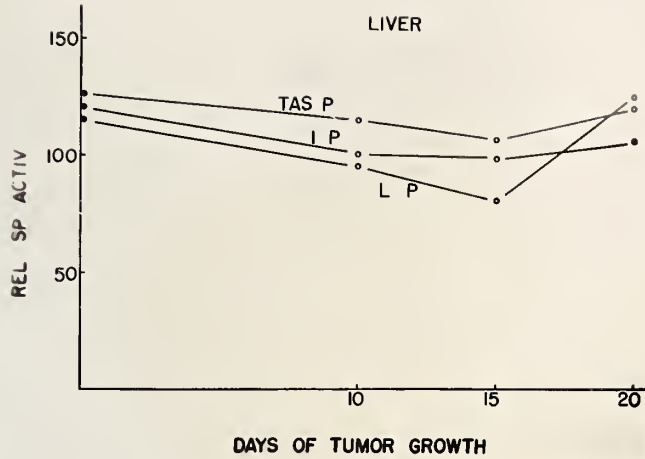


Fig. 24. The change in the relative specific activities of the inorganic P, total acid soluble P, and lipid P of the liver with time after tumor inoculation.

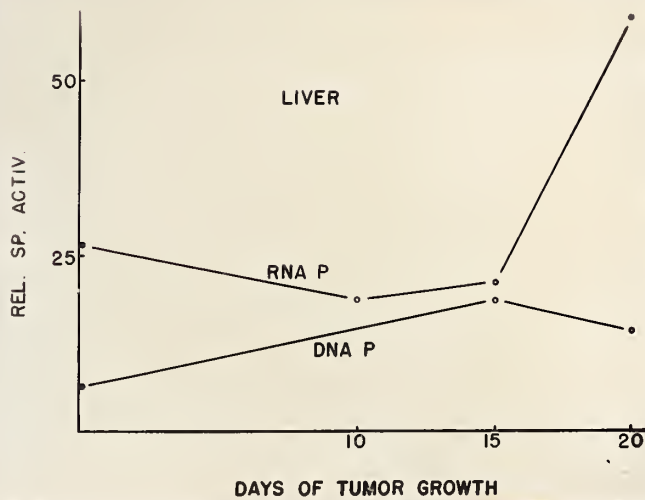


Fig. 25. The change in the relative specific activities of the RNA P and DNA P of the liver with time after tumor inoculation.

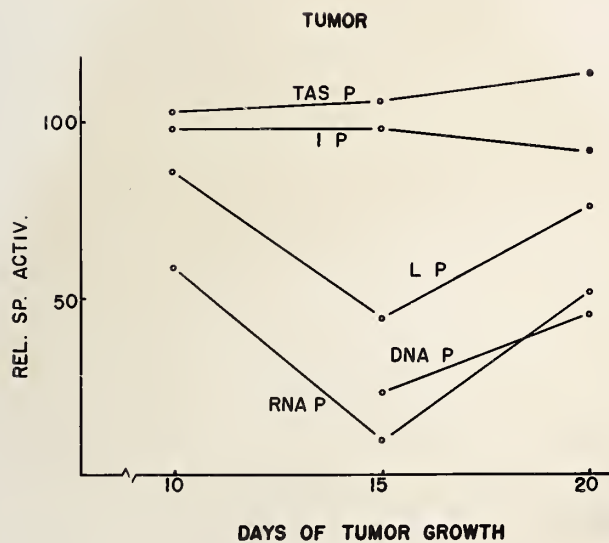


Fig. 26. The change in the relative specific activities of the inorganic P, total acid soluble P, lipid P, RNA P, and DNA P of the tumor with time after tumor innoculation.

specific activity of the DNA P at 20 days would indicate that the tumor was still growing rapidly.

C. EFFECTS OF CORTISONE AND ACTH

If there is a deficiency of Cortisone or ACTH in the tumor animal administration of these hormones might be expected to produce a partial recovery in the altered relative specific activities. This study was an attempt to determine whether such was the case. The animals were used 15 days after tumor innoculation and 8 hours after P³² injection.

1. Plasma

Neither cortisone nor ACTH produced any change in the corrected specific activities of the plasma inorganic P.

2. Adrenals

Cortisone produced a slight lowering in the relative specific activity of the inorganic P and total acid soluble P, the latter significant when compared to tumor animals with no hormone (Fig. 27). None of the other differences were statistically significant.

3. Liver

None of the differences between tumor and tumor plus hormone animals were significant except the slight decreases in the relative specific activities of the inorganic P and lipid P produced by ACTH (Fig. 28). The rise in the relative specific activities of the nucleic acids of all tumor animals as compared to normal animals is well shown.

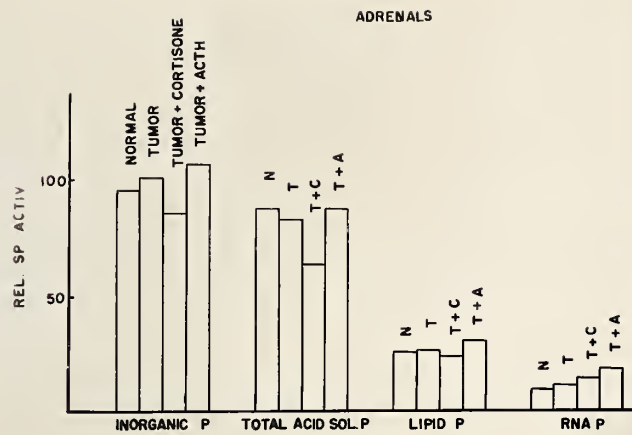


Fig. 27. The effect of Cortisone and ACTH on the relative specific activities of the adrenal tissue fractions.

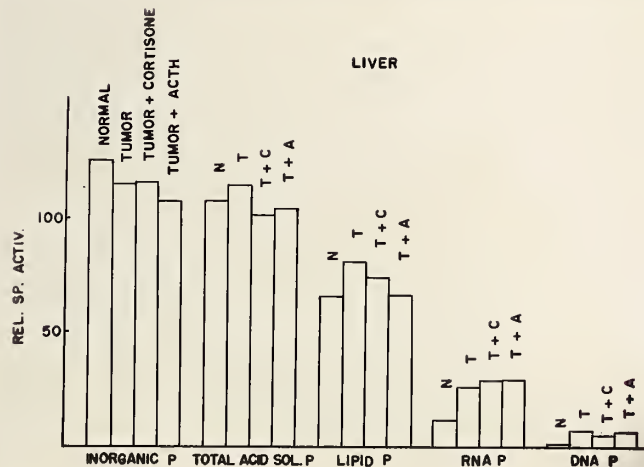


Fig. 28. The effect of Cortisone and ACTH on the relative specific activities of the liver tissue fractions.

D. AUTORADIOGRAPHS

The nature of the autoradiographs made inclusion of photographs impossible. However, it was noticed that there was a decreased action on the film in the centre of the tissue slice, supporting the finding of Gemzell (24) that only 10% of the P^{32} incorporated into the adrenal is found in the medulla.

The unavailability of stripping film, recommended for autoradiographs using P^{32} , made the use of X-ray film necessary. The emulsion is impregnated throughout the entire thickness of the film, causing a dispersion of the action of the P^{32} . For this reason the films could not be examined microscopically.

DISCUSSION

A comparison of the present investigation with the work of others (84, 25) reveals a close agreement in the P concentrations and relative specific activities in normal animals. All fractions show this correlation, with the exception of the adrenal RNA where a lower relative specific activity was obtained than that of Riedel (84). He found a relative specific activity of approximately 65 at 16 hours after P³² administration while the present investigation indicated a value of about 35 at the same interval. Such a discrepancy could arise either from a contamination of RNA by more active fractions in Riedel's work or by contamination with less active fractions in the present study.

The methods of extraction of the nucleic acids were different. Riedel used the extraction method proposed by Schmidt and Thannhauser (91) which was followed by purification using the procedure suggested by Hammarsten (35). The present study used the extraction procedure given by Hurlbert and Potter (44) and separation according to the method of Tyner et al (109). One of the problems associated with measurement of the nucleic acid activities is con-

tamination with a "phosphoprotein" fraction and it is possible that the two procedures removed relatively different amounts of this highly active fraction. Contamination of the RNA with the less active DNA might also be possible.

Other workers (20a) have compared the procedures used for separation of RNA with chromatography and electrophoresis and have found that there are significant differences in activity. Electrophoresis is now being applied to this work but as most of the results were obtained by the above methods it is not discussed here.

The changes found in the P metabolism of the tumor-bearing animals indicated no general disruption of the metabolism of the organs studied. Even at 20 days after tumor inoculation, when the animals were on the verge of death, the relative specific activities showed no great changes from normal. On the other hand, hypophysectomy has been shown to reduce the adrenal relative specific activities to half (25, 84) and cold stress caused a marked rise (76, 83).

A. PLASMA

No change in concentration or in corrected specific activity was noted in the plasma inorganic P when tumor-bearing animals were compared with normal animals. This is in marked contrast to the effect of hypophysectomy, where an increased P^{32} incorporation and decreased P con-

centration were noted (25, 84). There would therefore appear to be no alteration in transfer of P^{32} either out of or into the blood.

The stability of the plasma inorganic P permits the association of any changes in the activities in the organs with changes within the tissues themselves. The extracellular fluid is in rapid equilibrium with the plasma and probably immune to any biochemical changes in the organ (84). Therefore it may be postulated that there is no change in the amounts of P^{32} available to the cells and thus any changes in activities must be associated with biochemical changes within the cells themselves.

B. ADRENALS

The systemic changes in the host which were discussed previously might all be the result, at least in part, of either adrenal hyper- or hypo-activity. The anaemia, lowered liver catalase activity, and depressed glycogen-synthesizing ability of the liver have been associated with pituitary-adrenal hypofunction, while the thymus atrophy and hyperlipemia are suggested as indicating hyperactivity. This controversy can only mean that the adrenal in the tumor-bearing animal is not responsible for one of these two sets of reactions. It is possible to suppose that the tumor acts directly, or indirectly through some other organ, to produce one set of systemic effects, superseding the opposite influence of the adrenals. At the same

time the remaining set of systemic changes may be caused by an indirect action through the adrenals.

Although conclusive proof is lacking as yet, the investigations involving hypophysectomy and ACTH (25, 84) and cold stress (76, 83) would indicate that there is a correlation between pituitary stimulation of the adrenal cortex and its rate of P^{32} incorporation. If such is the case, the depressed activities of a number of the P-containing fractions would indicate that there was a hypoactivity of the gland. Whether this hypoactivity was a result of decreased pituitary stimulation, depressed sensitivity of the cortex to such stimulation, or some other factor cannot be determined from a study of this type. The further correlation of hypoactivity with hypofunction might also be postulated but cannot be shown.

The incorporation of P^{32} into the adrenal inorganic P was decreased, particularly 4 hours after P^{32} administration (Fig. 7). This difference was also found to occur in the total acid soluble P (Fig. 8), lipid P (Fig. 9), and RNA P (Fig. 10). The change was approximately the same in each case.

The inorganic P of the adrenal is a known precursor of these latter fractions and any change in its relative specific activity would result in a similar change in the activities of its products. Under these conditions, if the specific activities of the total acid soluble P, lipid P, and RNA P were calculated relative to that of the in-

organic P of the adrenal there would be a close correlation between tumor and normal animals. Since the adrenal inorganic P^{32} incorporation was decreased and no differences in the relative specific activities (calculated relative to the adrenal inorganic P) occurred in any of the other fractions studied, it is unlikely that there was any change in the P^{32} incorporation into the adrenals of tumor animals beyond a decreased transfer of P^{32} from the extracellular to the intracellular fluid.

Riedel (84) has postulated that the decreased P^{32} incorporation following hypophysectomy is due to a decreased transfer of P from the extracellular to the intracellular fluid space. The data obtained from tumor animals in the present investigation is not inconsistent with a similar hypothesis. The magnitude of the change, however, was not as great. This is reasonable, since it is unlikely that the tumor animal exhibits a complete lack of pituitary stimulation, as is the case with hypophysectomized animals.

The inability of ACTH administration to correct the decreased P^{32} incorporation into the adrenals of tumor animals is in contrast to the marked recovery obtained by Riedel in hypophysectomized animals (84). This might indicate that the adrenal changes were a direct result of the tumor on adrenal receptiveness to ACTH rather than a decrease in pituitary stimulation. On the other hand, it may only serve to stress further the inconsistency of conclusions drawn from the administration of exogenous hormones

to animals in which there has not been a removal of the endogenous supply.

If the hypothesis that the decreased P^{32} incorporation into the adrenals is associated with hypoactivity is correct, one might explain the anaemia and liver catalase and glycogen synthesis changes partially on the basis of a lack of adrenal stimulation. As previously noted, all of the difference cannot be attributed to cortical insufficiency, since even complete adrenalectomy will not produce as great a change as that found in the tumor-bearing animal.

Following this hypothesis further, the thymus changes were not due to adrenal hyperfunction and may have been the result of a direct action of the tumor. This would indicate that the "nitrogen trap" effect might be increased by a direct action of the tumor in mobilizing the protein stores of the host.

The thymus atrophy following inanition has been shown to be absent in both adrenalectomized and hypophysectomized animals (114). Thus the tumor-bearing animal would represent a special case. However, the thymus atrophy in tumor rats (but not mice (90)) is also dependent upon the adrenals (99). The fact that the adrenals are essential does not necessarily mean that the atrophy is a result of cortical hyperfunction, however. This problem deserves further investigation.

The extreme hyperlipemia noted in rats bearing the Walker 256 carcinoma (99) would also have to be explained

on the basis of a direct action of the tumor. Such a situation has already been postulated by Haven (36, 37).

The hypertrophy of the adrenals and the histological findings are in keeping with the theory of exhaustive hypofunction. Such a state would indicate a preliminary increase in the production of cortical hormones and, presumably, a rise in the P^{32} incorporation. Such a rise was not noted in tumor animals at 10 days, the earliest interval studied following tumor inoculation. Nicholls and Rossiter (76) noted an increased P^{32} incorporation even after 16 days of cold stress. The tumor has probably acted in some manner to depress the P^{32} incorporation into the adrenals in spite of a stress action which would have an opposite effect. The tumor was palpable and rapidly growing at 10 days. Whether there was a preliminary stress action resulting in an increased activity must await further investigation. A study of the adrenal gland at shorter intervals after tumor inoculation is indicated.

C. LIVER

The changes in the relative specific activities of the liver inorganic P, total acid soluble P, and lipid P bore no resemblance to the larger differences in the adrenals, indicating that the latter changes were probably a specific action on that organ. Conversely, the RNA P and DNA P changes in the liver were not found in the adrenals.

All of the fractions studied, with the exception of the RNA, incorporated P^{32} more rapidly than the corresponding fractions of the adrenals in normal animals. This is to be expected, since the liver is a very rapidly metabolizing organ.

The relative specific activities of the DNA P showed a marked increase over the normal values, an observation which has received much previous attention (55, 78). The magnitude of change was approximately the same as that observed by others. The increase has been ascribed to a hypertrophy of the liver, an increased demand for tissue synthesis, or a specific humor agent produced by rapidly dividing tissue. A similar increase has also been noted in the spleen and kidneys of tumorous rats (56).

The increased RNA P relative specific activity at the 8 hour interval in 15 day tumor animals and 16 hours after P^{32} in 20 day tumor animals is at variance with the results of other investigators (78, 16) who have found no change in the RNA P^{32} incorporation. A complete study of this problem was not attempted since the primary function of this investigation was to study changes in the adrenals. Further determinations of P^{32} incorporation into the liver are indicated.

D. TUMOR

A study of the relative specific activities of the various tumor fractions failed to indicate the increased rate of phosphorylation which has been postulated in tumor

tissue. To show the retention of P^{32} by the tumor it would be necessary to continue study beyond the 16 hour interval. A slight continuation of the increase in the total acid soluble P relative specific activity might indicate an increased rate of phosphorylation; this too would require study beyond 16 hours.

Investigations of the change in relative specific activities of the tumor fractions as a function of tumor age indicate a pronounced change in the lipid and nucleic acid fractions. Various stages in the growth of the tumor might be differentiated. Mider (69) has distinguished 3 stages in the growth of the tumor; the first in which the tumor grows slowly and the carcass gains weight, the second in which the tumor grows more rapidly and the host becomes anorectic with loss of weight, and the third in which the tumor grows slowly and the host's tissues rapidly lose nitrogen with excessive amounts of urea and nitrogen in the urine. Greenstein (33) has shown a biphasic effect of the tumor on liver catalase activity reduction. However, most of the evidence indicates that the metabolic changes in the tumor and the systemic changes in the host are a function of the tumor size.

SUMMARY

1. A study was made of the effect of the Walker 256 carcinoma on the P concentrations and P^{32} uptake of a number of P-containing fractions of the adrenals, liver, and tumor of the rat.
2. The P-containing fractions studied were the plasma inorganic P, tissue inorganic P, total acid soluble P, lipid P, RNA P, and DNA P.
3. Significant decreases were noted in the incorporation of P^{32} into the adrenal inorganic P, total acid soluble P, and lipid P of tumor-bearing animals. The changes were the greatest 4 hours after P^{32} administration and 15 days after tumor inoculation. It was suggested that the decreased incorporation of P^{32} in the adrenals might be due to a decreased transfer of P^{32} from the extracellular to the intracellular fluid space.
4. The adrenal changes were similar in direction, although not in magnitude, to the changes produced by hypophysectomy and opposite to the changes produced by cold stress.
5. A survey was made of the systemic changes that could be produced by adrenal cortical hyperfunction and

hypofunction. It was suggested that there was a hypofunction of the adrenal glands of tumor-bearing rats which was partially responsible for some of the systemic changes in the host.

6. Few changes were noted in the P concentrations of the fractions of all the tissues studied.
7. In a study of the P^{32} incorporation into the liver of the tumor-bearing rat, the typical increased activity of the DNA P was noted. Scattered changes were also noted in the P^{32} incorporation into the RNA.
8. A study of the P^{32} uptake in the P-containing fractions of the tumor indicated that there was a rapid rate of phosphorylation in tumor tissue as compared to liver tissue.
9. From a study of the changes in P^{32} incorporation as the tumor grew, the possibility of different phases in the growth of the tumor and in its systemic effects was commented upon.
10. ACTH and Cortisone were generally unable to alter significantly the relative specific activities of P concentrations in the liver and adrenal tissues of tumor animals.

BIBLIOGRAPHY

1. Adams, D. H., Brit. J. of Canc. 4: 183, 1950
2. Addis, T., Poo, L. J., and Lew, W., J. Biol. Chem. 115: 111, 1936
3. Albert, S. and Johnson, R. M., Canc. Res., 12: 584, 1952.
4. Albert, S., Johnson, R. M. and Cohan, M. S., Canc. Res. 11: 772, 1951
5. Annau, E., Manginelli, A. and Roth, A., Canc. Res. 11: 304, 1951
6. Appleman, D., Skavinski, E. R. and Stein, A. M., Canc. Res. 10: 498, 1950
7. Ball, H. A. and Samuels, L. T., Am. J. Canc. 32: 50, 1938
8. Ball, H. A. and Samuels, L. T., Proc. Soc. Exper. Biol. and Med. 38: 441, 1938
9. Baserga, R. and Shubik, P., Canc. Res. 14: 12, 1954
10. Begg, R. W., Canc. Res. 11: 341, 1951
11. Begg, R. W., Canc. Res. 11: 406, 1951
12. Begg, R. W. and Dickinson, T. E., Canc. Res. 11: 409, 1951
13. Begg, R. W. and Reynolds, E. F., Science 111: 721, 1950

14. Bhattacharya, K. L., Chakraborty, K. P., Bose, A.
and Das Gupta, N. N., Science 118; 65, 1953
15. Bloom, F., Proc. Soc. Exper. Biol. and Med. 79:
651, 1952
16. Brues, A. M., Tracy, M. M., and Cohn, W. E., J.
Biol. Chem. 155: 619, 1944
17. Burchenal, J. H., Stock, C. C. and Rhoads, C. P.,
Canc. Res. 10: 209, 1950
18. Conn, J. W. and Fajans, S. S., Ann. Rev. Physiol.
14: 453, 1952
19. Cori, C. F. and Cori, G. T., J. Biol. Chem. 65: 397,
1925
20. Dalton, A. J. and Peters, V. B., J. Nat. Canc. Inst.
5: 99, 1944
- 20a Davidson, J. N. and Smellie, R. M. S., Biochem. J.
52: 594, 1952
21. Dounce, A. L. and Shanewise, R. P., Canc. Res. 10:
103, 1950
22. Ernster, L., Zetterström, R. and Lindberg, O., Acta
chem. scand. 4: 942, 1950
23. Funk, C., Tomashefsky, P., Soukup, R. and Ehrlich, A.,
Brit. J. Canc. 5: 280, 1951
24. Gemzell, C. A., Acta Endocrinologica: supp.I, 1948
25. Gemzell, C. A. and Samuels, L. T., Endocrinology 47:
48, 1950
26. Gottschalk, R. G. and Grollman, A., Canc. Res. 12:
651, 1952

27. Greenfield, R. E. and Meister, A., Canc. Res. 10:
222, 1950
28. Greenfield, R. E. and Meister, A., J. Nat. Canc.
Inst. 11: 997, 1951
29. Greenstein, J. P., J. Nat. Canc. Inst. 2: 525, 1942
30. Greenstein, J. P., Biochemistry of Cancer, Academic
Press, Inc., N. Y., 1954
31. Greenstein, J. P. and Andervont, H. B., J. Nat. Canc.
Inst. 4: 283, 1943
32. Greenstein, J. P., Andervont, H. B. and Thompson, J.W.,
J. Nat. Canc. Inst. 2: 589, 1942
33. Greenstein, J. P., Jenrette, W. V. and White, J., J.
Nat. Canc. Inst. 2: 283, 1941
34. Griffin, A. C., Bloom, S., Cunningham, L., Teresi, J.
D. and Luck, J. M., Cancer 3: 316, 1950
35. Hammarsten, E., Acta med. scand. 128: supp. 196: 634,
1947
36. Haven, F. L., Bloor, W. R., and Randall, C., Canc.
Res. 9: 511, 1949
37. Haven, F. L., Bloor, W. R. and Randall, C., Canc.
Res. 11: 619, 1951
38. Hevesy, G., J. Chem. Soc. 1213, 1939
39. Hevesy, G., Radioactive Indicators, Interscience Pub-
lishers, Inc., N. Y., 1948
40. Holt, M. W. and Warren, S., Proc. Soc. Exper. Biol.
and Med. 76: 4, 1951
41. Holt, M. W., Cowing, R. F., and Warren, S., Science
110: 328, 1949

42. Homburger, F., Science 107: 648, 1948
43. Huggins, C. and Gergens, D. M., Canc. Res. 12: 134, 1952
44. Hurlbert, R. B. and Potter, V. R., J. Biol. Chem. 195: 257, 1952
45. Ingle, D. J., Proc. Soc. Exper. Biol. and Med. 38: 443, 1938
46. Ingle, D. J., Endocrinology 37: 7, 1945
47. Ingle, D. J. and Baker, B. L., Endocrinology 48: 313, 1951
48. Ingle, D. J. and Nezamis, J. E., Endocrinology 48: 484, 1951
49. Ingle, D. J., Prestrud, M. C. and Li, C. H., Am. J. Physiol. 166: 165, 1951
50. Ingle, D. J., Prestrud, M. C. and Nezamis, J. E., Am. J. Physiol. 166: 171, 1951
51. Ingle, D. J., Prestrud, M. C. and Rice, K. L., Endocrinology 46: 510, 1950
52. Jones, H. B., Chaikoff, I. L. and Lawrence, J. H., Am. J. Cancer 40: 243, 1940
53. Kahler, H. and Robertson, W. B., J. Nat. Canc. Inst. 3: 495, 1943
54. Kamen, M. D., Radioactive Tracers in Biology, Academic Press, N. Y., 1951
55. Kelly, L. S. and Jones, H. B., Science 111: 333, 1950
56. Kelly, L. S., Payne, A. H., White, M. R. and Jones, H. B., Canc. Res. 11: 694, 1951
57. Koletsky, S., Bonte, F. J. and Friedell, H. L., Canc. Res. 10: 129, 1950

58. LePage, G. A., *Canc. Res.* 8: 193, 1948
59. Loeb, L. and Kirtz, M. M., *Am. J. Canc.* 36: 56, 1939
60. Loefer, J. B., *Cancer* 5: 161, 1952
61. Long, C. N. H., *Recent Prog. in Hormone Res.* 1: 99, 1947
62. Long, C. N. H., Katzin, B. and Fry, E. G., *Endocrinology* 26: 309, 1940
63. McEuen, C. S. and Thomson, D. L., *Brit. J. Exper. Path.* 14: 384, 1933
64. McEuen, C. S. and Selye, H., *Am. J. Med. Sci.* 189: 423, 1935
65. McEwen, H. D. and Haven, F. L., *Canc. Res.* 1: 148, 1941
66. Mider, G. B., *Canc. Res.* 11: 821, 1951
67. Mider, G. B., *Ann. Rev. Med.* 4: 187, 1953
68. Mider, G. B., Fenninger, L. D., Haven, F. L. and Morton, J. J., *Canc. Res.* 11: 731, 1951
69. Mider, G. B., Tesluk, H. and Morton, J. J., *Acta Unio Intern contre Cancrum* 6: 409, 1948
70. Miller, L. L., *J. Biol. Chem.* 172: 113, 1948
71. Moon, H. D., *Proc. Soc. Exper. Biol. and Med.* 37: 34, 1937
72. Murphy, J. B. and Sturm, E., *Canc. Res.* 7: 417, 1947
73. Murphy, J. B. and Sturm, E., *Canc. Res.* 8: 139, 1948
74. Nakahara, W. and Fukuoka, F., *Gann* 40: 45, 1949
75. Nakahara, W. and Fukuoka, F., *Gann* 41: 47, 1950
76. Nicholls, D. and Rossiter, R. J., *Chem. in Canada* 5: 104, 1953
77. Payne, A. H., Kelly, L. S. and Jones, H. B., *Canc. Res.* 12: 666, 1952

78. Payne, A. H., Kelly, L. S., and White, M. R., *Canc. Res.* 12: 65, 1952
79. Pearson, O. H., Eliel, L. P. and Rawson, R. W., *Proc. 1st Clinical ACTH Conf.*: 318, 1950
80. Poo, L. J., Lew, W., Lee, D. D. and Addis, T., *J. of Nutrition* 19: 505, 1940
81. Potter, V. R. and Elvehjem, C. A., *J. Biol. Chem.* 114: 495, 1936
82. Rainwater, L. J. and Wu, C. S., *Nucleonics* 1: 60, 1947
83. Reiss, M. and Halkerston, J. M., *J. Endocrinol.* 6: 369, 1950
84. Riedel, B. E., *Effect of Hypophysectomy and the administration of ACTH on the Phosphorus Metabolism of the Adrenal and the Liver of the Rat*, Ph.D. Thesis, Univ. of Western Ontario, 1953
85. Sacks, J. and Altshuler, C. H., *Am. J. Physiol.* 137: 750, 1942
86. Samuels, L. T. and Ball, H. A., *Am. J. Canc.* 18: 380, 1933
87. Sarason, E. L., *Arch. Int. Med.* 71: 702, 1943
88. Sayers, G. and Sayers, M. A., *Recent Prog. in Hormone Res.* 2: 81, 1948
89. Savard, K., *Science* 108: 381, 1948
90. Savard, K. and Homburger, F., *Proc. Soc. Exper. Biol. and Med.* 70: 68, 1949
91. Schmidt, G. and Thannhauser, S. J., *J. Biol. Chem.* 149: 425, 1943

92. Schneider, W. C., J. Biol. Chem. 161: 293, 1945
93. Schrek, R., Am. J. Canc. 24: 807, 1935
94. Selye, H., Brit. J. Exper. Path. 17: 234, 1936
95. Selye, H., J. Clin. Endocrin. 6: 117, 1946
96. Sherman, C. D., Morton, J. J. and Mider, G. B., Canc. Res. 10: 374, 1950
97. Smith, M. C., Slattey, P. A., Shimkin, M. B., Li, C. H., Lee, R., Clarke, J. C. and Lyons, W. R., Canc. Res. 12: 59, 1952
98. Stetten, DeW., Recent Prog. in Hormone Res. 4: 189, 1949
99. Stewart, A. G., Systemic Effects of Malignant Tumors, Ph.D. Thesis, Univ. of Western Ontario, 1952
100. Stewart, A. G. and Begg, R. W., Canc. Res. 13: 556, 1953
101. Sturm, E. and Murphy, J. B., Canc. Res. 4: 384, 1944
102. Sugiura, K., Stock, C. C., Dobriner, K. and Rhoads, C. P., Canc. Res. 10: 244, 1950
103. Talalay, P., Takano, G. M. V. and Huggins, C., Canc. Res. 12: 834, 1952
104. Talalay, P., Takano, G. V. M. and Huggins, C., Canc. Res. 12: 838, 1952
105. Taylor, A. and Pollack, M. A., Canc. Res. 2: 223, 1942
106. Tepperman, J., Engel, F. L. and Long, C. N. H., Endocrinology 32: 403, 1943
107. Thorn, G. W. and Forsham, P. H., Recent Prog. in Hormone Res. 4: 229, 1949
108. Tourtellotte, W. W. and Storer, J. B., Canc. Res. 10: 783, 1950

109. Tyner, E. P., Heidelberger, C. and LePage, G. A., Canc. Res. 13: 186, 1953
110. Warburg, O., Abhandl. deut. Akad. Wiss. Berlin 3: 1947
111. Wenner, C. E., Spirtes, M. A. and Weinhouse, S., Canc. Res. 12: 44, 1952
112. Wenner, C. E. and Weinhouse, S., Canc. Res. 13: 21, 1953
113. White, A. and Dougherty, T. F., Endocrinology 36: 16, 1945
114. White, A. and Dougherty, T. F., Endocrinology 41: 230, 1947
115. Young, N. F., Abels, J. C. and Homburger, F., J. Clin. Investigation 27: 760, 1948
116. Young, N. F., Kensler, C. J., Seki, L. and Homburger, F., Proc. Soc. Exper. Biol. and Med. 66: 322, 1947
117. Zilversmit, D. B., Entenman, C. and Fishler, M. C., J. Gen. Physiol. 26: 325, 1943

APPENDIX A

EXPERIMENTAL PROCEDURE

Animals and Tissues

1. Remove food, but not water, from animals 24 hours before killing.
2. Inject P^{32} at appropriate interval before killing, use the same syringe to inject one dose of P^{32} into a 25 mil volumetric flask for the P^{32} standard.
3. Remove from anaesthesia after breathing has ceased but before heart has stopped beating. Immediately make a longitudinal incision along the abdomen and into the pleural cavity.
4. Remove approximately 5 mls of blood from the aorta in a heparinized syringe. Transfer to a 15 mil centrifuge tube through the open barrel of the syringe.
5. Remove the adrenals, clear of adhering adipose and connective tissue, and immediately freeze in liquid nitrogen. Keep frozen until homogenization.
6. Remove approximately 200 - 300 mgm. of liver from the central lobe, blot, freeze. Keep frozen.
7. Remove 200 - 300 mgm. of tumor tissue from the periphery of the tumor. Avoid necrotic and capsular tissue. Blot, freeze. Keep frozen.
8. Weigh the tissues.

Plasma Inorganic P

1. Remove blood sample to cold room (3°C.) as soon as possible. Centrifuge for 10 minutes.
2. Transfer 200 microlitres (μ l) to a second 15 mil centrifuge tube.
3. Add 3 mils of 10% trichloroacetic acid (TCA), stir well. Let stand for 30 minutes.
4. Centrifuge, transfer supernatant to 10 mil volumetric flask.
5. Wash twice with 3 mils of 10% TCA, add to 10 mil volumetric. Bring the flask up to volume, shake.
6. Take a 5 mil aliquot and add to a test tube containing
 - 5.0 mils butanol-benzene
 - 0.8 mils perchloric acid
 - 0.5 mils ammonium molybdate solution
7. Shake for 30 seconds.
8. Remove 3 mils of the organic (upper) layer for P estimation.

Tissue Determinations

1. Homogenize in 3 (5)* mils of 10% TCA in the cold room, transfer to a 15 (50) mil centrifuge tube.
2. Centrifuge and remove supernatant to a 10 (25) mil volumetric flask.

*Throughout the procedures the bracketed figures and instructions refer to the liver and tumor determinations, as distinguished from the adrenal determinations.

3. Wash precipitate 4 (6) times with 2.5 (5) mls of 10% TCA. Add first 2 washes to the volumetric, discard the others.
4. Bring the flask up to volume, shake. Use this fraction for the inorganic P, ATP P, and total acid soluble P determinations.
5. Add 5 (10) mls of ethyl alcohol to the precipitate. Reserve for the lipid extractions.

Inorganic P

Must be done immediately and while still in the cold room.

1. Add 3 mil aliquot to a test tube containing
 - 2.0 mls water
 - 5.0 mls butanol-benzene
 - 0.8 mls perchloric acid
 - 0.5 mls ammonium molybdate solution
2. Allow to stand 3 minutes, shake for 30 seconds.
3. Remove 3 mls of the organic layer for P estimation.

ATP P

1. Transfer 3 mil aliquot to test tube, add 0.8 mls perchloric acid.
2. Hydrolyse for 20 minutes at 100°C.
3. Cool, add
 - 2.0 mls water
 - 5.0 mls butanol-benzene
 - 0.5 mls ammonium molybdate solution

3. Shake for 30 seconds.
4. Remove 3 mils of organic layer for P estimation.

Total Acid Soluble P

1. Transfer 3 (1) mil aliquot to a 10 mil Kjeldahl flask, add 1.0 mil perchloric acid.
2. Evaporate, ash for 10 minutes.
3. Transfer to a test tube using 5 mils of water in two portions. Add
 - 5.0 mils butanol-benzene
 - 0.5 mils ammonium molybdate solution
4. Shake for 30 seconds.
5. Remove 3 mils of organic layer for P estimation.

Lipid P

1. Wash the precipitate from the acid soluble P extraction twice with 5 (10) mils of cold ethyl alcohol. Transfer supernatant to 25 (100) mil volumetric flask.
2. Boil the precipitate for 3 minutes in 4 (5) portions of 3 (5) mils of alcohol-ether mixture. Transfer the first 3 (all) washes to volumetric.
3. Wash with 5 (10) mils of ether. Transfer to volumetric.
4. Bring the volumetric up to volume with ether (alcohol-ether mixture), shake.
5. Transfer 10 (5) mil aliquot to a 10 mil Kjeldahl flask, add 1.0 mil perchloric acid.

6. Evaporate, ash for 10 minutes.
7. Transfer to test tube with 5 mls of water in two portions. Add
 - 5.0 mls butanol-benzene
 - 0.5 mls ammonium molybdate solution
7. Shake for 30 seconds.
8. Remove 3 mls of organic layer for P estimation.

RNA P

1. Extract the residue from the lipid extraction with 3 (10) mls, then 2 (5) mls, of 10% sodium chloride at 100°C. Transfer supernatants to 15 (50) ml centrifuge tube.
2. Add 10 (30) mls alcohol, place in cold room overnight.
3. Centrifuge, discard supernatant. Wash precipitate two times with 2 (5) mls cold alcohol, once with 2 mls of ether. Discard washings.
4. Add 1 (2) mls of 0.1N sodium hydroxide, hydrolyse for 18 hours at 37°C.
5. Place in cold room, add 0.1 (0.2) mls concentrated HCl.
6. Centrifuge, transfer supernatant to Kjeldahl flask. Wash precipitate twice with 1 ml dilute HCl and add to flask.
7. Add 1.0 ml perchloric acid, evaporate, ash for 10 minutes.
8. Transfer to test tube using 5 mls of water in two portions.

9. Add 5.0 mils butanol-benzene
0.5 mils ammonium molybdate solution
10. Shake for 30 seconds.
11. Remove 3 (1) mils of organic layer for P estimation.

DNA P (Liver and Tumor samples only)

1. Dissolve precipitate from step #6 of RNA P in 3 mils of 0.1N sodium hydroxide.
2. Transfer to 10 mil Kjeldahl flask. Wash centrifuge tube twice with 1 mil 0.1N sodium hydroxide and add to flask.
3. Add 1.0 mil perchloric acid, evaporate, ash for 10 minutes.
4. Transfer to test tube using 5 mils water in two portions. Add

5.0 mils butanol-benzene

0.5 mils ammonium molybdate solution

5. Shake for 30 seconds.
6. Remove 3 mils of organic layer for P estimation.

P Estimation and P³² Determination

1. Place the aliquot from the organic layer in a 10 mil volumetric flask, add 3 mils (5 mils in the case of liver RNA P) of acid-alcohol mixture.
2. Add 0.5 mils of dilute stannous chloride solution, shake.
3. Allow to stand 25 minutes, read the optical density in a spectrophotometer at 730 mμ.

4. The blank consists of an equal quantity of the solvent used in the particular determination (10% TCA, alcohol-ether, dilute HCl, or 0.1N sodium hydroxide) which has been carried through the procedure.
5. The P standard consists of 1.0 mil of dilute P standard solution plus 4 mils water, treated in the same manner as the samples, beginning at the step where the butanol-benzene is added.
6. After P estimation, bring the 10 mil volumetric flasks up to volume with acid-alcohol mixture. Transfer to an M-6 liquid Geiger-Müller counter tube. Count for 3 minutes, or 1 minute in the case of samples with a counting rate over 800 impulses per minute.

P³² Standard

1. Bring the 25 mil volumetric flask from step #2 of Animals and Tissues up to volume with water, shake.
2. Transfer three 25 microlitre aliquots to planchettes.
3. Count for 3 minutes, average. Use to correct for variations of P³² dosage from experiment to experiment.

Reagents

1. 10% trichloroacetic acid in water.
2. Butanol-benzene mixture. Equal parts by volume of isobutanol and benzene.
3. 60% perchloric acid.
4. 10% ammonium molybdate solution in water.
5. Alcohol-ether mixture. 3 parts by volume of alcohol, 1 part ethyl ether.

6. 10% sodium chloride solution in water.
7. Acid-alcohol mixture. 32 mls of concentrated sulfuric acid dissolved in 968 mls of alcohol.
8. Concentrated stannous chloride solution. 10.0 grams $\text{SnCl}_2 \cdot 2\text{H}_2\text{O}$ dissolved in 25.0 mls concentrated HCl . Keep in cold room.
9. Dilute stannous chloride solution. 0.25 mls of concentrated stannous chloride solution dissolved in 50 mls 1N sulfuric acid. Prepare fresh before using.
10. Concentrated P standard. 2.194 grams KH_2PO_4 , add to 500 mls with water. Add a few drops of chloroform as a preservative, keep in the cold room.
11. Dilute P standard. 1.0 ml concentrated P standard, add to 100 mls with water.

APPENDIX B

TABLE I

Plasma Inorganic P - Normal Animals

Effect of Time after Administration
of P^{32} on P^{32} Incorporation

Time in Hours

2		4		8		16	
S/A	Corr.S/A	S/A	Corr.S/A	S/A	Corr.S/A	S/A	Corr.S/A
423	83.3	424	70.8	324	66.0	128	40.4
279	58.6	414	66.3	295	76.0	123	43.2
522	151.4	402	75.1	353	71.0	134	43.4
563	163.3	339	67.9	315	60.1	165	41.6
375	80.6	372	67.0	350	78.1	166	45.3
439	137.0			353	78.7	184	46.4
837	150.7			307	85.7	158	48.3
682	103.7			273	89.0	162	47.3
				237	70.6	124	23.8
				175	60.4	133	27.5
				198	59.0	136	30.1
				284	89.5	132	30.8
						188	29.5
						134	25.7
						169	31.3
						156	25.1
						148	25.2
						118	28.0
						151	25.1
						154	29.4
						163	56.4
						119	40.9
						188	54.9
Mean	515 116.1	390 69.4		289 73.7		149 36.7	
S.E.M.	8.75	1.44		9.62		2.14	

TABLE II

Plasma Inorganic P - 15 Day Tumor Animals

Effect of Time after Administration
of P^{32} on P^{32} Incorporation

Time in Hours							
2		4		8		16	
S/A	Corr.S/A	S/A	Corr.S/A	S/A	Corr.S/A	S/A	Corr.S/A
399	99	375	86.1	338	72.6	152	26.4
477	98	357	64.3	276	69.3	162	29.0
370	133	417	78.4	278	86.2	134	25.0
601	168	378	77.6	239	74.1	185	36.4
337	115	392	75.3	223	70.9		
460	162	390	74.9				
369	126	383	74.2				
529	157						
424	117						
387	125						
529	109						
673	116						
467	96						
628	104						
522	107						
531	104						
Mean	481 121	385 75.8		271 74.6		158 29.3	
S.E.M.	5.59	2.27		2.69		2.15	

TABLE III

Plasma Inorganic P - 16 Hour P³²

Effect of Time after Tumor Inoculation
on P Concentration and P³² Incorporation

Time in Days

10			15			20		
P mgm. %	S/A	Corr. S/A	P mgm. %	S/A	Corr. S/A	P mgm. %	S/A	Corr. S/A
8.77	183	33.5	9.33	152	26.4	9.48	148	29.5
7.23	177	34.9	10.87	162	29.0	9.00	126	26.1
8.88	181	31.1	7.68	134	25.5	8.15	136	36.2
5.95	221	37.3	16.90	185	36.4	7.23	147	29.7
7.22	162	30.8				8.90	128	36.2
5.44	165	32.3				4.06	276	48.9
7.90	162	29.0				5.86	172	50.4
7.34	179	32.7	11.19	158	29.3	7.53	162	36.7
1.46		0.98	1.74		2.15	0.69		3.35

TABLE IV

Plasma Inorganic P - 8 Hour P³² and 15 Day Tumor

Effect of Cortisone and ACTH on P Concentration and
P³² Incorporation

Tumor				Tumor + Cortisone			Tumor + ACTH		
P mgm. %	S/A	Corr. S/A		P mgm. %	S/A	Corr. S/A	P mgm. %	S/A	Corr. S/A
				7.08	303	74.2	8.58	260	70.3
				6.60	266	62.3	8.82	246	69.3
				5.81	249	64.3	6.09	283	66.4
				7.74	292	89.6	6.95	278	84.8
				6.23	248	78.9	6.51	286	82.7
				8.89	262	79.4			
Mean	5.76	271	74.6	7.06	270	74.8	7.39	271	74.7
S.E.M.	0.17		2.69	0.42		3.81	0.49		3.37

Data for tumor animals from Table II

APPENDIX C

TABLE I

Adrenal Inorganic P - Normal Animals

Effect of Time after Administration
of P^{32} on P^{32} Incorporation

Time in Hours							
2		4		8		16	
S/A	Rel.S/A	S/A	Rel.S/A	S/A	Rel.S/A	S/A	Rel.S/A
243	57.4	682	161	216	66.8	190	148
208	74.5	642	155	257	87.1	190	154
435	83.3	544	136	353	100.1	187	140
749	133.0	484	143	335	106.5	251	152
401	106.9	405	109	383	109.3	226	136
344	78.4			314	89.1	323	176
444	53.0			267	87.1	277	175
463	67.9			214	78.3	302	186
				224	94.3	193	156
				175	100.2	258	187
				210	106.2	300	221
				290	102.1	127	96
						218	116
						124	93
						139	82
						187	120
						158	107
						123	104
						196	130
						154	100
						150	92
						119	100
						164	87
Mean	411 81.8	552 141		270 93.9		198 133	
S.E.M.	2.78	8.14		2.82		7.81	

TABLE II

Adrenal Inorganic P - 15 Day Tumor

Effect of Time after Administration
of P^{32} on P^{32} Incorporation

Time in Hours

2		4		8		16	
S/A	Rel.S/A	S/A	Rel.S/A	S/A	Rel.S/A	S/A	Rel.S/A
237	59.4	367	98.1	219	64.9	202	132.9
435	91.2	346	97.0	298	108.0	152	93.8
219	59.2	388	92.9	303	109.1	128	95.5
364	60.6	373	98.7	254	106.4	213	115.1
342	101.5	421	107.4	244	109.2		
410	89.1	312	80.1				
261	70.7	427	111.6				
283	53.5						
255	60.1						
206	53.2						
523	98.9						
623	92.6						
367	78.6						
451	71.8						
374	71.6						
428	80.6						
Mean	361 74.5	376 97.8	264 99.5	174 109.3			
S.E.M.	3.94	3.56	7.75	7.99			

TABLE III

Adrenal Total Acid Soluble P - Normal Animals

Effect of Time after Administration
of P^{32} on P^{32} Incorporation

Time in Hours

2		4		8		16	
S/A	Rel.S/A	S/A	Rel.S/A	S/A	Rel.S/A	S/A	Rel.S/A
223	52.7	507	119	160	49.4	187	146
155	55.6	497	120	154	52.3	175	142
207	39.6	421	105	241	68.3	174	130
329	58.4	348	103	292	92.8	206	125
280	74.7	375	101	327	93.5	219	132
276	62.9			245	69.3	259	141
351	41.9			225	73.2	234	148
420	61.6			227	83.3	231	143
				260	109.6	175	141
				183	104.3	177	128
				257	130.0	143	105
				301	106.1	173	131
						152	81
						157	117
						160	95
						185	119
						154	104
						135	114
						221	146
						161	105
						193	119
						134	113
						171	91
Mean	280 55.9	430 110		239 86.0		182 122	
S.E.M.	3.78	3.57		6.76		5.34	

TABLE IV

Adrenal Total Acid Soluble P - 15 Day Tumor

Effect of Time after Administration
of P³² on P³² Incorporation

Time in Hours

2		4		8		16	
S/A	Rel.S/A	S/A	Rel.S/A	S/A	Rel.S/A	S/A	Rel.S/A
161	40.3	334	89.2	144	42.6	207	136
193	40.5	276	77.3	230	83.3	159	98
148	40.0	274	65.7	266	95.8	135	100
186	30.9	339	89.6	198	82.6	230	124
181	53.7	314	80.1	221	99.0		
206	44.8	255	65.4				
217	58.8	366	95.7				
218	41.2						
185	43.6						
152	39.3						
424	63.0						
273	58.5						
296	47.1						
264	50.6						
300	56.5						
Mean	227 47.3	308 80.4		212 80.7		189 115	
S.E.M.	2.29	4.16		8.70		8.04	

TABLE V

Adrenal Lipid P - Normal Animals

Effect of Time after Administration
of P³² on P³² Incorporation

Time in Hours							
2		4		8		16	
S/A	Rel.S/A	S/A	Rel.S/A	S/A	Rel.S/A	S/A	Rel.S/A
8.9	1.9	78.8	18.6	41.9	12.9	100	78.1
5.5	2.0	65.8	15.9	45.9	15.6	94	76.4
18.2	3.5	72.2	18.0	75.5	21.4	109	81.3
18.9	3.4	66.1	19.5	82.9	26.3	133	80.6
12.5	3.3	51.7	13.9	102.7	29.3	112	67.5
25.8	5.9			57.1	16.2	140	76.1
21.7	2.6			57.5	18.7	130	82.3
25.9	3.8			73.8	31.1	148	91.4
				57.5	32.9	134	108.1
				67.7	34.2	136	99.6
				112.8	39.7	105	77.2
						140	106.1
						106	56.4
						74	55.2
						100	59.2
						114	73.1
						79	53.4
						86	72.9
						124	82.0
						84	55.0
						103	63.1
						70	58.5
						90	47.8
Mean	17.7 3.3	66.9 17.2		70.5 25.3		109 73.9	
S.E.M.	0.42	0.90		2.54		3.42	

TABLE VI

Adrenal Lipid P - 15 Day Tumor

Effect of Time after Administration
of P³² on P³² Incorporation

Time in Hours

2		4		8		16	
S/A	Rel.S/A	S/A	Rel.S/A	S/A	Rel.S/A	S/A	Rel.S/A
6.2	1.5	51.4	13.7	45.8	13.6	121	79
8.5	1.8	20.0	5.6	59.7	21.6	95	55
6.3	1.7	19.0	4.5	80.5	29.0	83	62
7.9	1.3	44.4	11.7	66.3	27.7	119	64
7.0	2.1	25.8	6.6	79.8	35.8		
7.6	1.6	27.7	7.1				
8.9	2.4	32.9	8.6				
11.5	2.2						
7.5	1.8						
5.8	1.5						
18.6	3.5						
28.3	4.2						
16.1	3.5						
21.6	3.4						
19.1	3.7						
14.3	2.7						
Mean	12.2 2.4	31.6 8.3		66.4 25.5		105 65	
S.E.M.	0.07	1.17		3.35		4.37	

TABLE VII

Adrenal RNA P - Normal Animals

Effect of Time after Administration
of P^{32} on P^{32} Incorporation

Time in Hours					
4		8		16	
S/A	Rel.S/A	S/A	Rel.S/A	S/A	Rel.S/A
39.8	9.4	14.2	4.4	85	63
44.7	10.8	8.8	3.0	100	61
38.1	9.5	25.0	7.1	112	68
22.8	6.7	58.6	18.6	151	82
24.4	6.5	47.2	13.5	95	60
		4.8	1.4	120	74
		12.3	4.0	52	38
		23.8	10.0	52	28
		16.8	9.6	41	30
		16.7	8.4	47	28
		66.9	23.6	83	53
				49	33
				50	42
				58	39
				17	11
				33	20
				33	28
				36	19
Mean	33.9 8.6	26.8 9.4		67	33
S.E.M.	0.74	1.97			4.76

TABLE VIII

Adrenal RNA P - 15 Day Tumor

Effect of Time after Administration
of P^{32} on P^{32} Incorporation

Time in Hours

	2		4		8		16	
	S/A	Rel.S/A	S/A	Rel.S/A	S/A	Rel.S/A	S/A	Rel.S/A
	9.5	2.6	41.1	11.0	26.7	9.7	54.8	36
	16.0	4.7	29.3	8.2	29.3	10.5	47.9	29
	15.7	3.4	26.0	6.2	19.0	7.9	26.3	19
	9.5	1.8	21.4	5.6	32.9	14.7	48.6	27
	10.6	1.6	16.3	4.2				
	4.2	0.9	21.5	5.5				
	7.1	1.1	29.8	7.8				
	7.7	1.5						
	11.5	2.2						
Mean	10.2	2.2	26.5	6.9	27.0	10.7	44.4	28
S.E.M.		0.38		0.79		1.25		3.03

TABLE IX

Adrenal Inorganic P - 16 Hour P^{32} Effect of Time after Tumor Innoculation
on P Concentration and P^{32} Incorporation

Time in Days

	10			15			20		
	P mgm. %	S/A	Rel. S/A	P mgm. %	S/A	Rel. S/A	P mgm. %	S/A	Rel. S/A
	35.6	171	93.4				32.9	162	109
	35.8	149	84.2				38.6	163	129
	35.0	165	91.2				28.6	155	114
	31.0	200	90.5				25.4	194	132
	32.0	147	90.7				35.0	116	91
	28.5	155	93.9				21.2	195	71
	41.5	126	77.8				22.6	180	105
Mean	34.2	159	88.8	27.5	174	109	29.2	166	107
S.E.M.	1.48		2.01	2.68		7.99	2.30		7.44

Data for 15 day tumor animals from Table II

TABLE X

Adrenal Total Acid Soluble P - 16 Hours P³²

Effect of Time after Tumor Inoculation
on P Concentration and P³² Incorporation

Time in Days									
10			15			20			
P mgm. %	S/A	Rel. S/A	P mgm. %	S/A	Rel. S/A	P mgm. %	S/A	Rel. S/A	
80.6	203	111				86.0	143	105	
81.9	174	98				92.5	173	117	
84.0	202	112				141.4	164	130	
76.8	199	90				90.8	176	120	
76.8	183	113				98.4	140	109	
83.1	171	104				91.5	184	67	
77.4	184	114				101.2	166	97	
Mean	80.1	188	106	76.4	183	115	100.3	164	106
S.E.M	1.08	3.19	4.76		8.04	6.58		7.15	

Data for 15 day tumor animals from Table IV

TABLE XI

Adrenal Lipid P - 16 Hours P³²

Effect of Time after Tumor Innoculation
on P Concentration and P³² Incorporation

Time in Days									
10			15			20			
P mgm. %	S/A	Rel. S/A	P mgm. %	S/A	Rel. S/A	P mgm. %	S/A	Rel. S/A	
112.6	102	63.0				108.3	103	69.6	
100.7	111	60.6				162.5	117	92.9	
107.1	104	58.8				99.7	105	77.2	
122.7	118	65.2				115.9	133	90.5	
106.7	109	49.3				128.5	88	68.8	
93.5	115	69.7				117.5	109	39.5	
100.5	100	61.7				130.1	110	64.0	
Mean	106.2	108	61.2	114.3	105	65.0	123.2	109	71.8
S.E.M.	3.31	2.21	5.96		4.37	7.11		6.29	

Data for 15 day tumor animals from Table VI

TABLE XII

Adrenal RNA P - 16 Hours P³²

Effect of Time after Tumor Innoculation
on P Concentration and P³² Incorporation

Time in Days									
10			15			20			
P mgm.%	S/A	Rel. S/A	P mgm.%	S/A	Rel. S/A	P mgm.%	S/A	Rel. S/A	
11.09	60.6	33.1				9.14	52.2	35.3	
9.36	49.6	28.0				4.60	63.4	50.3	
3.16	82.2	45.4				10.43	51.9	38.2	
1.85	66.7	30.2				6.60	68.0	46.3	
5.52	58.2	35.9				18.51	59.1	46.2	
18.20	44.2	26.8				15.54	60.2	21.8	
13.90	39.2	24.2				6.99	56.9	33.1	
Mean	9.01	57.2	31.9	9.89	44.4	27.7	10.30	58.8	38.7
S.E.M.	2.07	2.48	1.16		3.03	1.77		3.43	

Data for 15 day tumor animals from Table VIII

TABLE XIII

Adrenal Inorganic P - 8 Hour P³² & 15 Day Tumor

Effect of Cortisone and ACTH on
P Concentration and P³² Incorporation

	Tumor			Tumor + Cortisone			Tumor + ACTH		
	P mgm.%	S/A	Rel. S/A	P mgm.%	S/A	Rel. S/A	P mgm.%	S/A	Rel. S/A
				12.1	178	58.6	25.5	255	98.1
				19.1	209	78.6	22.9	266	107.9
				16.0	158	63.5	18.0	271	95.9
				26.0	258	88.3	17.3	309	111.2
				18.8	271	109.4	25.1	324	113.1
				23.3	277	105.6			
Mean	19.7	264	99.5	19.2	225	84.0	21.8	285	105.2
S.E.M.	1.22		7.75	1.85		7.86	1.55		3.11

Data for tumor animals from Table II

TABLE XIV

Adrenal Total Acid Soluble P - 8 Hour P³² & 15 Day Tumor

Effect of Cortisone and ACTH on

P Concentration and P³² Incorporation

	Tumor			Tumor + Cortisone			Tumor + ACTH		
	P		Rel.	P		Rel.	P		Rel.
	mgm. %	S/A	S/A	mgm. %	S/A	S/A	mgm. %	S/A	S/A
				62.9	111	36.6	86.3	186	71.4
				82.4	176	66.1	88.2	207	84.0
				81.3	118	47.4	91.1	190	67.2
				79.9	211	72.3	67.6	282	101.5
				79.8	233	94.0	80.8	305	106.5
				86.2	149	57.0			
Mean	81.0	212	80.7	78.7	167	62.2	82.8	234	86.1
S.E.M.	3.03		8.70	3.03		7.51	3.72		7.02

Data for tumor animals from Table IV

TABLE XV

Adrenal Lipid P - 8 Hour P³² & 15 Day Tumor

Effect of Cortisone and ACTH on

P Concentration and P³² Incorporation

Tumor			Tumor + Cortisone			Tumor + ACTH		
P mgm. %	S/A	Rel. S/A	P mgm. %	S/A	Rel. S/A	P mgm. %	S/A	Rel. S/A
			136	40.8	15.3	119	53.8	20.7
			99	36.6	14.7	125	78.3	31.8
			114	71.9	24.6	144	53.1	18.9
			121	82.5	33.3	85	95.5	34.4
			126	73.9	28.2	109	129.1	45.1
Mean	122	66.4 25.5	119	61.1	23.2	116	82.0	30.2
S.E.M.	7.03	3.35	5.45		3.25	8.61		4.29

Data for tumor animals from Table VI

TABLE XVI

Adrenal RNA P - 8 Hour P³² & 15 Day Tumor

Effect of Cortisone and ACTH on
P Concentration and P³² Incorporation

	Tumor			Tumor + Cortisone			Tumor + ACTH		
	P mgm. %	S/A	Rel. S/A	P mgm. %	S/A	Rel. S/A	P mgm. %	S/A	Rel. S/A
				3.48	21.6	8.1	4.07	21.2	8.1
				2.14	45.0	18.1	7.57	50.9	20.7
				7.03	24.6	8.42	4.38	50.9	18.0
				8.99	44.7	18.0	2.47	47.1	16.9
				11.93	47.5	18.1	7.08	76.5	26.8
Mean	8.70	27.0	10.7	6.71	36.7	14.2	5.11	49.3	18.1
S.E.M.	1.78		1.25	1.60		2.15	0.86		2.70

Data for tumor animals from Table VIII

APPENDIX D

TABLE I

Liver Inorganic P - Normal Animals

Effect of Time after Administration
of P³² on P³² Incorporation

Time in Hours							
2		4		8		16	
S/A	Rel.S/A	S/A	Rel.S/A	S/A	Rel.S/A	S/A	Rel.S/A
480	114	527	124	378	117	183	143
317	114	548	132	355	121	151	123
806	154	583	145	448	127	171	128
685	122	463	136	353	112	242	147
721	192	410	110	470	134	229	138
832	190			539	153	249	135
886	106			463	151	226	143
947	139			279	102	240	148
				242	102	166	134
				191	109	154	112
				237	120	183	135
				329	116	144	109
						223	119
						136	102
						149	94
						181	116
						153	103
						131	111
						165	109
						146	95
						181	111
						128	108
						209	111
Mean	709 141	506 130		357 122		180 121	
S.E.M.	11.38	5.32		4.62		3.43	

TABLE II

Liver Inorganic P - 15 Day Tumor

Effect of Time after Administration
of P^{32} on P^{32} Incorporation

Time in Hours

2		4		8		16	
S/A	Rel.S/A	S/A	Rel.S/A	S/A	Rel.S/A	S/A	Rel.S/A
431	125	463	124	386	114	146	96
414	104	494	139	324	118	149	92
411	86	548	131	293	106	142	106
444	120	495	131	268	112	191	103
562	94	508	130	248	111		
513	112	504	129				
524	142	502	132				
757	143						
615	145						
622	161						
628	119						
878	131						
704	151						
788	126						
471	90						
700	132						
Mean	591 124	502 131		303 112		157 99	
S.E.M.	5.38	1.52		1.80		2.78	

TABLE III

Liver Total Acid Soluble P - Normal Animals

Effect of Time after Administration
of P32 on P32 Incorporation

Time in Hours

2		4		8		16	
S/A	Rel.S/A	S/A	Rel.S/A	S/A	Rel.S/A	S/A	Rel.S/A
478	113	471	111	370	114	180	141
307	110	470	113	327	111	143	116
478	92	502	125	386	109	175	131
500	89	369	109	277	88	223	135
464	124	449	121	377	108	215	130
557	127			383	109	246	134
661	79			340	111	219	139
727	107			272	100	202	125
				212	89	194	156
				199	114	178	129
				211	107	167	123
				289	102	187	142
						251	134
						172	128
						191	113
						219	140
						163	110
						131	111
						179	119
						148	96
						200	123
						145	122
						216	115
Mean	522 105	452 116		304 105		189 127	
S.E.M.	5.66	2.73		2.42		2.70	

TABLE IV

Liver Total Acid Soluble P - 15 Day Tumor Animals

Effect of Time after Administration
of P³² on P³² Incorporation

Time in Hours

2		4		8		16	
S/A	Rel.S/A	S/A	Rel.S/A	S/A	Rel.S/A	S/A	Rel.S/A
374	94	444	119	345	102	166	109
397	83	436	122	293	106	168	103
320	87	481	115	314	113	148	110
475	79	467	123	280	117	196	106
312	93	479	122	272	122		
403	88	383	98				
381	103	474	124				
515	97						
428	101						
446	115						
558	106						
719	107						
541	116						
632	101						
529	101						
574	108						
Mean	475 99	452 118		301 112		170 107	
S.E.M.	2.62	3.18		3.20		1.37	

TABLE V

Liver Lipid P - Normal Animals

Effect of Time after Administration
of P³² on P³² Incorporation

Time in Hours

2		4		8		16	
S/A	Rel.S/A	S/A	Rel.S/A	S/A	Rel.S/A	S/A	Rel.S/A
81.5	19.3	179	42.1	212	65.5	139	109
68.4	24.5	146	35.2	194	66.0	124	101
62.6	12.0	174	43.3	240	68.0	141	105
73.6	13.1	65	19.2	197	62.6	179	108
55.8	14.9	63	17.0	233	66.5	165	99
87.0	19.8			233	66.0	200	109
225.5	26.6			221	72.1	175	111
128.9	18.9			164	60.1	206	127
				138	58.4	208	168
				126	71.7	206	149
				115	58.0	203	149
				172	60.5	189	143
						283	155
						151	113
						178	105
						172	110
						152	103
						120	102
						147	97
						126	82
						154	95
						135	114
						202	107
Mean	97.9 18.6	125 31.4		187 64.6		172 115	
S.E.M.	1.72	5.01		1.32		4.44	

TABLE VI

Liver Lipid P - 15 Day Tumor Animals

Effect of Time after Administration
of P³² on P³² Incorporation

		Time in Hours							
		2		4		8		16	
		S/A	Rel.S/A	S/A	Rel.S/A	S/A	Rel.S/A	S/A	Rel.S/A
		21.0	5.3	136	36.3	230	68.0	92	61
		19.8	4.1	121	33.7	193	69.9	158	99
		37.9	10.2	134	32.1	216	77.6	112	83
		25.1	4.2	141	37.3	215	89.7	151	81
		38.7	11.5	63	16.2	205	91.8		
		31.6	6.9	73	18.8				
		43.7	11.8	118	30.8				
		55.3	10.4						
		63.7	15.0						
		74.0	19.1						
		83.2	15.7						
		112.2	16.7						
		100.0	21.4						
		94.7	15.1						
		97.2	18.6						
		85.3	16.1						
Mean		61.5	12.6	112	29.3	212	79.4	128	81
S.E.M.			1.32		2.94		4.40		6.74

TABLE VII

Liver RNA P - Normal Animals

Effect of Time after Administration
of P³² on P³² Incorporation

Time in Hours							
2		4		8		16	
S/A	Rel.S/A	S/A	Rel.S/A	S/A	Rel.S/A	S/A	Rel.S/A
17.6	3.4	28.6	6.7	39.6	12.2	25.7	20.1
11.0	1.9	29.2	7.0	41.2	14.0	18.7	15.2
59.2	15.8	20.0	5.0	56.2	15.9	20.4	15.2
41.8	9.5	16.9	5.0	32.9	10.5	33.3	20.2
16.6	2.0	10.6	2.8	58.4	16.7	34.0	20.5
14.8	2.2			44.3	12.6	42.0	22.8
				39.8	13.0	34.6	21.9
				25.7	9.4	38.8	24.0
				19.9	8.4	57.6	46.5
				20.5	11.7	56.0	40.6
				18.8	9.5	44.8	32.9
				25.9	9.1	90.5	68.6
						111.8	59.5
						29.2	21.8
						28.1	16.6
						33.9	21.7
						27.5	18.6
						15.4	13.1
						33.5	22.0
						22.0	14.0
						37.8	23.2
						27.2	22.9
						51.4	27.3
Mean	26.8 5.8	21.1 5.3		35.3 11.9		39.8 26.5	
S.E.M.	2.12	0.67		0.74		2.90	

TABLE VIII

Liver RNA P - 15 Day Tumor Animals

Effect of Time after Administration
of P³² on P³² Incorporation

Time in Hours

	2		4		8		16	
	S/A	Rel.S/A	S/A	Rel.S/A	S/A	Rel.S/A	S/A	Rel.S/A
	10.9	2.9	43.8	11.7	45.2	13.4	25.8	17
	14.0	2.3	55.4	15.5	49.7	11.0	32.6	20
	11.5	3.4	35.8	8.6	88.9	32.0	39.1	29
	11.4	2.5	19.3	5.1	76.6	32.1	38.4	21
	22.6	6.1	26.5	6.8	76.0	34.1		
	10.1	1.9	18.6	4.8				
	46.6	11.0	24.5	6.4				
	41.1	10.6						
	11.9	2.3						
	16.1	2.4						
	11.7	2.5						
	16.6	2.6						
	10.5	2.0						
	10.8	2.0						
Mean	17.6	3.9	32.0	8.4	67.3	25.9	34.0	22
S.E.M.		0.80		1.37		3.80		2.22

TABLE IX

Liver DNA P - Normal Animals

Effect of Time after Administration
of P³² on P³² Incorporation

		Time in Hours							
		2		4		8		16	
		S/A	Rel.S/A	S/A	Rel.S/A	S/A	Rel.S/A	S/A	Rel.S/A
		2.9	0.7	6.3	1.5	5.2	1.61	6.5	5.2
		1.1	0.2	3.4	0.8	6.6	2.23	3.3	2.4
				4.1	1.0	6.5	1.84	7.5	5.5
				4.1	1.2	5.0	1.58	5.9	4.5
				10.4	2.8	7.9	2.26	34.6	18.4
						5.8	1.64	11.1	7.4
						4.5	1.47	2.3	1.5
						6.9	2.53	3.9	2.4
						3.0	1.26	6.5	5.5
						5.0	2.87	20.5	10.9
						0.8	0.38		
						3.9	1.37		
Mean		2.0	0.45	5.7	1.5	5.1	1.75	10.2	6.4
S.E.M.			0.36		0.31		0.18		1.51

TABLE X

Liver DNA P - 15 Day Tumor Animals

Effect of Time after Administration
of P³² on P³² Incorporation

		Time in Hours							
		2		4		8		16	
		S/A	Rel.S/A	S/A	Rel.S/A	S/A	Rel.S/A	S/A	Rel.S/A
		3.1	0.8	18.2	4.8	36.0	10.66	36.1	23
		0.4	0.1	14.8	4.1	20.5	7.44	37.9	23
		5.0	1.5	20.6	4.9	18.6	6.69	14.1	11
		1.9	0.5	54.2	14.3	8.1	3.39	40.4	22
		1.1	0.2	13.4	3.4	16.1	7.22		
		0.9	0.2	8.7	2.2				
		3.4	0.9	25.4	6.6				
Mean		2.3	0.59	22.2	5.8	19.9	7.08	32.1	20
S.E.M.			0.17		1.40		1.03		2.54

TABLE XI

Liver Inorganic P - 16 Hour P³²

Effect of Time after Tumor Innoculation
on P Concentration and P³² Incorporation

Time in Days									
10			15			20			
P mgm. %	S/A	Rel. S/A	P mgm. %	S/A	Rel. S/A	P mgm. %	S/A	Rel. S/A	
46.4	185	101				34.4	169	114	
46.5	178	101				31.4	158	125	
48.9	174	96				36.8	153	113	
38.9	192	87				32.4	185	126	
51.9	181	112				42.0	129	101	
44.5	174	106				29.7	186	67	
50.3	168	104				32.6	177	103	
Mean	46.8	179	101	28.4	157	99	34.2	165	107
S.E.M.	1.30		2.77	1.50		2.78	1.44		7.03

Data for 15 day tumor animals from Table II

TABLE XII

Liver Total Acid Soluble P - 16 Hour P^{32}

Effect of Time after Tumor Innoculation
on P Concentration and P^{32} Incorporation

Time in Days									
10			15			20			
P mgm. %	S/A	Rel. S/A	P mgm. %	S/A	Rel. S/A	P mgm. %	S/A	Rel. S/A	
74.4	219	120				84.7	182	123	
86.5	188	106				92.4	180	143	
91.2	174	96				98.7	164	121	
86.2	247	112				100.1	198	135	
97.4	210	130				101.9	142	111	
89.7	196	119				96.0	208	75	
90.3	199	123				90.5	231	134	
Mean	87.9	205	115	63.3	170	107	94.9	186	120
S.E.M.	2.45	1.26	2.93		1.37	2.12		7.91	

Data for 15 day tumor animals from Table IV

TABLE XIII

Liver Lipid P - 16 Hour P³²

Effect of Time after Tumor Innoculation
on P Concentration and P³² Incorporation

Time in Days									
10			15			20			
P mgm. %	S/A	Rel. S/A	P mgm. %	S/A	Rel. S/A	P mgm. %	S/A	Rel. S/A	
113	173	94.5				96	191	129	
122	173	97.7				110	188	149	
108	166	91.7				115	162	119	
123	205	92.8				116	228	155	
128	164	101.2				121	202	73	
123	149	90.3				110	231	134	
106	155	95.7							
Mean	117	169	95.0	114	128	81.0	112	239	127
S.E.M.	3.03	4.25	1.99		6.74	2.80		10.4	

Data for 15 day tumor animals from Table VI

TABLE XIV

Liver RNA P - 16 Hour P³²

Effect of Time after Tumor Innoculation
on P Concentration and P³² Incorporation

Time in Days

	10			15			20		
	P mgm. %	S/A	Rel. S/A	P mgm. %	S/A	Rel. S/A	P mgm. %	S/A	Rel. S/A
	35.2	50.5	27.6				35.0	66.1	44.7
	43.6	40.6	22.9				25.3	84.3	66.9
	45.5	27.0	14.9				22.3	80.1	58.9
	41.5	42.9	19.4				36.1	62.1	42.2
	50.0	26.6	16.4				36.4	88.6	69.2
	43.4	25.5	15.5				26.6	122.2	44.3
	43.3	26.5	16.4				25.9	150.9	87.7
Mean	43.2	34.2	19.0	56.6	34.0	21.8	29.7	93.5	59.1
S.E.M.	1.56		1.64	1.31		2.22	2.09		5.88

Data for 15 day tumor animals from Table VIII

TABLE XV

Liver DNA P - 16 Hour P³²

Effect of Time after Tumor Innoculation
on P Concentration and P³² Incorporation

Time in Days						
15				20		
P			Rel.	P		Rel.
mgm. %	S/A		S/A	mgm. %	S/A	S/A
				12.83	24.6	16.6
				9.43	14.8	11.7
				5.70	5.1	3.7
				6.55	8.6	5.9
				3.58	41.9	32.7
				7.57	22.8	8.3
				5.66	42.2	24.5
Mean	13.89	32.1	19.8	7.33	22.9	14.8
S.E.M.	0.66		2.54	1.06		11.13

Data for 15 day tumor animals from Table X

TABLE XVI

Liver Inorganic P - 8 Hour P³² & 15 Day Tumor

Effect of Cortisone and ACTH on
P Concentration and P³² Incorporation

Tumor			Tumor + Cortisone			Tumor + ACTH		
P mgm. %	S/A	Rel. S/A	P mgm. %	S/A	Rel. S/A	P mgm. %	S/A	Rel. S/A
			27.8	374	123	33.4	248	95
			25.8	329	124	32.1	267	108
			26.1	299	120	27.1	319	113
			29.8	293	100	28.7	291	105
			23.3	269	108	27.3	302	106
			26.0	272	104			
Mean	30.1	303 112	26.5	306	113	29.9	285	105
S.E.M.	1.16	1.80	0.82		3.83	1.13		2.50

Data for tumor animals from Table II

TABLE XVII

Liver Total Acid Soluble P - 8 Hour P³² & 15 Day Tumor

Effect of Cortisone and ACTH on
P Concentration and P³² Incorporation

	Tumor			Tumor + Cortisone			Tumor + ACTH		
	P mgm. %	S/A	Rel. S/A	P mgm. %	S/A	Rel. S/A	P mgm. %	S/A	Rel. S/A
				88.4	357	118	89.1	231	89
				98.8	279	105	79.1	241	98
				101.8	274	110	100.1	293	104
				96.1	293	100	100.1	289	104
				96.4	271	109	96.7	338	118
				82.0	287	110			
Mean	96.9	301	112	93.9	294	109	93.0	278	102
S.E.M.	1.32		3.2	2.73		2.18	3.60		4.31

Data for tumor animals from Table IV

TABLE XVIII

Liver Lipid P - 8 Hour P³² & 15 Day Tumor

Effect of Cortisone and ACTH

P Concentration and P³² Incorporation

	Tumor			Tumor + Cortisone			Tumor + ACTH		
	P mgm. %	S/A	Rel. S/A	P mgm. %	S/A	Rel. S/A	P mgm. %	S/A	Rel. S/A
				123	230	75.9	97	119	45.6
				133	181	67.8	107	114	46.1
				119	164	65.9	133	198	70.0
				118	176	60.3	121	234	84.2
				121	200	80.7	126	237	82.9
				117	220	84.1			
Mean	126	212	79.4	122	195	72.5	117	180	65.8
S.E.M.	1.89		4.40	2.16		3.45	5.84		4.13

Data for tumor animals from Table VI

TABLE XIX

Liver RNA P - 8 Hour P³² & 15 Day Tumor

Effect of Cortisone and ACTH on
P Concentration and P³² Incorporation

	Tumor			Tumor + Cortisone			Tumor + ACTH		
	P		Rel.	P		Rel.	P		Rel.
	mgm. %	S/A	S/A	mgm. %	S/A	S/A	mgm. %	S/A	S/A
				33.8	55.8	18.4	43.3	43.0	16.5
				60.0	47.2	17.7	38.4	44.2	17.9
				56.5	43.4	17.4	50.0	41.5	14.7
				26.3	135.0	46.2	27.1	136.7	49.2
				28.9	94.2	38.0	29.9	140.4	49.1
				25.4	97.5	37.2			
	Mean	36.8	67.3	25.9	38.5	78.9	29.2	37.7	81.2
S.E.M.	1.95	3.80	5.83		4.77	3.78		6.89	

Data for tumor animals from Table VIII

TABLE XX

Liver DNA P - 8 Hour P³² & 15 Day Tumor

Effect of Cortisone and ACTH on
P Concentration and P³² Incorporation

	Tumor			Tumor + Cortisone			Tumor + ACTH		
	P		Rel.	P		Rel.	P		Rel.
	mgm. %	S/A	S/A	mgm. %	S/A	S/A	mgm. %	S/A	S/A
				6.18	5.6	1.83	9.61	13.3	5.09
				11.09	12.4	4.64	7.83	9.3	3.78
				9.11	10.9	4.36	8.98	16.2	5.72
				7.80	16.2	5.55	6.00	30.8	11.08
				7.62	15.7	6.33	7.77	26.4	9.23
				5.91	29.0	11.07			
Mean	7.41	19.9	7.08	7.95	14.9	5.63	8.04	19.2	6.98
S.E.M.	0.76		1.03	0.72		1.14	0.55		1.24

Data for tumor animals from Table X

APPENDIX E

TABLE I

Tumor Inorganic P - 15 Day Tumor Animals

Effect of Time after Administration
of P³² on P³² Incorporation

Time in Hours

2		4		8		16	
S/A	Rel.S/A	S/A	Rel.S/A	S/A	Rel.S/A	S/A	Rel.S/A
145	39.2	220	58.6	145	67.8	194	128
146	24.3	352	98.7	258	104.0	171	106
162	48.1	378	94.0	172	98.3	143	107
208	45.2	355	120.1	192	85.0	102	55
110	29.8	230	58.6	148	70.5		
308	58.2	265	68.0	195	121.9		
111	26.2	445	116.4	168	81.2		
308	79.6			127	92.7		
108	20.4						
149	22.1						
261	55.9						
261	41.6						
83	15.9						
249	46.9						
Mean	186 39.5	335 87.8		176 90.2		153 99	
S.E.M.	4.58	9.16		5.95		13.34	

TABLE II

Tumor Total Acid Soluble P - 15 Day Tumor Animals

Effect of Time after Administration
of P³² on P³² Incorporation

Time in Hours

2		4		8		16	
S/A	Rel.S/A	S/A	Rel.S/A	S/A	Rel.S/A	S/A	Rel.S/A
128	34.6	220	58.6	177	82.7	197	130
129	21.5	316	88.5	259	104.4	180	111
132	39.2	355	85.1	191	109.1	159	119
142	31.0	434	109.9	230	101.8	119	64
115	31.2	168	42.9	165	78.6		
246	46.5	244	62.5	223	139.4		
112	26.4	415	108.4	161	77.8		
237	61.2			143	104.4		
131	24.8						
159	23.6						
218	46.9						
298	47.4						
87	16.7						
297	55.9						
Mean	174 36.2	307 80.1	194 99.8	164 106			
S.E.M.	3.50	9.31	6.76	12.58			

TABLE III

Tumor Lipid P - 15 Day Tumor Animals

Effect of Time after Administration
of P³² on P³² Incorporation

Time in Hours

	2		4		8		16	
	S/A	Rel.S/A	S/A	Rel.S/A	S/A	Rel.S/A	S/A	Rel.S/A
	3.2	0.8	26.4	7.1	89.1	41.6	84	55
	2.7	0.7	40.2	11.3	80.1	32.3	51	31
	2.3	0.4	29.1	7.0	74.1	42.3	90	67
	1.5	0.4	39.5	10.4	64.9	28.7	47	25
	1.0	0.2	10.3	2.6	60.5	28.8		
	3.1	0.7	44.8	11.5	76.5	47.8		
	5.8	1.5	44.0	11.5	38.2	18.5		
					45.4	33.1		
Mean	2.8	0.7	33.5	8.8	66.1	34.1	68	45
S.E.M.		0.15		1.18		3.10		8.58

TABLE IV

Tumor RNA P - 15 Day Tumor Animals

Effect of Time after Administration
of P³² on P³² Incorporation

Time in Hours

2			4			8			16		
S/A	Rel.S/A		S/A	Rel.S/A		S/A	Rel.S/A		S/A	Rel.S/A	
3.1	0.8		27.2	7.3		62.6	29.3		5.3	3.4	
1.1	0.2		32.4	9.1		70.1	28.3		30.8	19.0	
3.5	1.0		33.5	8.0		66.0	37.7		7.9	5.9	
5.1	1.1		40.5	10.7		57.6	27.4		28.9	15.6	
4.1	1.1		9.5	2.4		58.0	36.3				
15.2	2.9		45.8	11.7		24.2	11.7				
4.0	0.9		38.8	10.1		39.8	29.1				
14.5	3.7										
Mean	4.6	1.5	32.5	8.5		54.0	28.5		18.2	11.0	
S.E.M.		0.39		1.08			2.96			3.23	

TABLE V

Tumor DNA P - 15 Day Tumor Animals

Effect of Time after Administration
of P³² on P³² Incorporation

Time in Hours

	2		4		8		16	
	S/A	Rel.S/A	S/A	Rel.S/A	S/A	Rel.S/A	S/A	Rel.S/A
	0.4	0.1	18.8	5.0	43.5	20.3	48.0	31.6
	0.7	0.2	29.8	8.3	81.8	33.0	16.6	10.3
	1.3	0.3	20.2	4.8	49.0	28.0	55.5	41.4
	5.3	1.0	22.5	5.9	47.4	22.6	23.7	12.8
	0.3	0.1	4.1	1.0	48.0	30.0		
	6.4	1.6	35.9	9.2	23.7	11.4		
			21.4	5.6	48.4	35.3		
Mean	2.4	0.5	21.8	5.7	48.4	25.8	36.0	24.0
S.E.M.		0.23		0.92		2.90		6.49

TABLE VI

Tumor Inorganic P - 16 Hour P32

Effect of Time after Tumor Innoculation
on P Concentration and P32 Incorporation

Time in Days									
10			15			20			
P mgm. %	S/A	Rel. S/A	P mgm. %	S/A	Rel. S/A	P mgm. %	S/A	Rel. S/A	
65.9	144	79				44.3	183	124	
58.0	102	58				41.2	64	51	
43.7	184	102				45.2	125	92	
48.6	216	98				37.1	156	106	
47.1	201	124				44.0	113	88	
35.2	192	116				34.6	200	72	
32.3	176	109				35.8	201	117	
Mean	47.3	174	98	41.1	153	99	40.3	149	93
S.E.M.	4.16		7.95	6.22		13.34	1.55		8.96

Data for 15 day tumor animals from Table I

TABLE VII

Tumor Total Acid Soluble P - 16 Hour P³²Effect of Time after Tumor Innoculation
on P Concentration and P³² Incorporation

Time in Days									
10			15			20			
P mgm. %	S/A	Rel. S/A	P mgm. %	S/A	Rel. S/A	P mgm. %	S/A	Rel. S/A	
94.5	175	96				94.6	185	125	
110.9	117	66				109.9	62	49	
76.3	204	113				99.3	131	96	
90.7	236	107				37.8	382	260	
81.2	212	131				92.6	132	103	
86.7	215	130				93.3	184	70	
61.5	202	125				91.6	178	103	
Mean	86.0	194	110	72.0	164	106	88.4	179	115
S.E.M.	5.40		8.10	6.15		12.58	8.12		24.08

Data for 15 day tumor animals from Table II

TABLE VIII

Tumor Lipid P - 16 Hour P³²

Effect of Time after Tumor Innoculation
on P Concentration and P³² Incorporation

Time in Days									
10			15			20			
P mgm. %	S/A	Rel. S/A	P mgm. %	S/A	Rel. S/A	P mgm. %	S/A	Rel. S/A	
44.1	150	82.0				54.4	169	114.2	
37.3	101	57.1				31.1	37	29.4	
35.7	120	66.3				47.5	130	95.6	
48.1	214	96.8				46.0	133	90.5	
29.8	176	108.6				39.8	102	79.7	
54.9	176	107.0				56.6	152	55.1	
39.2	135	83.3							
Mean	41.3	153	85.8	57.5	68	44.5	45.9	121	77.4
S.E.M.	2.94		6.85	5.19		8.58	3.53		11.38

Data for 15 day tumor animals from Table III

TABLE IX

Tumor RNA P - 16 Hour P³²

Effect of Time after Tumor Innoculation
on P Concentration and P³² Incorporation

Time in Days

	10			15			20		
	P mgm. %	S/A	Rel. S/A	P mgm. %	S/A	Rel. S/A	P mgm. %	S/A	Rel. S/A
	34.9	132	71.9				43.8	103	69.7
	18.9	104	58.7				11.8	22	17.1
	47.1	76	41.8				45.7	87	63.8
	44.8	130	58.7				28.3	75	51.2
	44.4	128	78.7				20.6	67	52.3
	57.1	94	56.7				33.4	178	64.3
	46.5	75	46.1						
Mean	42.2	105	58.9	48.8	18	11.0	31.6	89	53.1
S.E.M.	4.21		4.56	6.90		3.23	4.32		7.10

Data for 15 day tumor animals from Table IV

TABLE X

Tumor DNA P - 16 Hour P³²

Effect of Time after Tumor Innoculation
on P Concentration and P³² Incorporation

Time in Days						
15			20			
	P mgm. %	S/A	Rel. S/A	P mgm. %	S/A	Rel. S/A
				23.1	83.0	56.1
				2.3	27.9	22.1
				20.9	75.0	55.1
				20.8	66.5	45.2
				12.9	63.3	49.5
				18.2	113.6	41.2
				21.9	93.4	54.3
Mean	23.0	35.9	24.0	17.2	74.7	46.2
S.E.M.	3.53		6.49	2.57		4.19

Data for 15 day tumor animals from Table V

B29770